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BONE-MUSCLE RELATIONSHIPS IN BEEF CARCASSES

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BONE development in cattle has long been a controversial issue among cattle producers, feeders, buyers, processors and consumers. Breeding cattle are often criticized for being too light of bone and slaughter steers are complimented for possessing quality and refinement of bone. Information is needed concerning size of bone as an indicator of muscle development, since the composition of beef carcasses is becoming increasingly important to all segments of the beef cattle industry. Accurate criteria are needed for estimating muscling in live cattle. Hammond (1921, 1932), McMeekan (1940, 1942, 1956), Palsson (1939) and Walker (1958) have completely dissected thousands of meat animals and have reported a strong positive correlation between the weight of bone in each animal and the weight of muscle tissue. The purpose of this study was to determine the weight and length relationships between certain long bones of cattle and the association between bone thickness and muscling.

Experimental Procedure

Detailed slaughter, carcass and bone data were obtained on 28 yearling Hereford steers, representing six lines of breeding, which were fed for 215 days, averaged 919 lb. at slaughter, and yielded carcasses that graded from U.S. Standard to U.S. Choice. The slaughter procedure was essentially as recommended by Deans (1951). Both sides of the carcasses were cut by procedures described by Wellington (1953).

The metacarpus, metatarsus, tibia, femur and ulna-radius were cleaned of all ligaments, tendons, fat and other extraneous matter and weighed and measured for length in a manner described by Wythe (1958).

The chuck, rib, loin and round were given a "retail trim" as described by King *et al.* (1959) so that all fat in excess of ¼ in. and excess bony portions were removed. The weights of these cuts, as well as area of ribeye muscle (*Longissimus dorsi* at 12th rib) were used as indicators of muscling. Weight-length ratios of the various bones were determined by dividing the weight of each bone by its length.

Results and Discussion

Ranges and means of chilled carcass weights, bone weights, bone lengths, various beef cut weights and area of ribeye muscles are presented in table 1.

Inheritance fundamentally determines the potential development of both bone and muscle characteristics. In a study of beef and dual purpose Shorthorns, Hankins *et al.* (1943) found muscle:bone ratio to be a rather definitely inherited characteristic. Nutrition certainly has some influence on the relative development of bone and muscle. Weseli *et al.* (1958) indicated that steers scored as having larger bones tended slightly to have lower dressing percents and less external fat thickness. Lush (1926) reported little or no evidence of any relation between dressing percentage and percentage of bone when the degree of fatness was constant. Since muscles are attached to bones and their chief function is to operate and move bones, it seems logical that muscle size and shape may be associated with the size and shape of bones. However, Weseli *et al.* (1958), Merkel (1958) and Arthaud (1958) have reported low partial correlation coefficients (carcass weight held con-

TABLE 1. RANGES AND MEANS OF CHILLED CARCASS WEIGHTS, BONE WEIGHTS, BONE LENGTHS, VARIOUS BEEF CUT WEIGHTS AND AREA OF RIBEYE MUSCLE

Item	Range	Mean
Chilled carcass wt., lb.	426.0 –715.0	559.0
Sum loin, rib, round and rump, lb.	100.8 –161.6	130.4
Sum "retail trimmed" chuck, rib and loin, lb.	81.3 –131.3	103.6
"Retail trimmed" boneless cushion round and rump, lb.	35.8 – 53.5	43.3
Area ribeye muscle at 12th rib, sq. in.	8.1 – 11.5	9.5
Metacarpus weight, lb.	0.67– 1.035	0.851
Metacarpus length, cm.	17.20– 21.60	19.56
Tibia weight, lb.	1.89– 2.95	2.37
Tibia length, cm.	29.70– 36.35	32.97

TABLE 2. CORRELATION COEFFICIENTS BETWEEN THE WEIGHTS AND BETWEEN THE LENGTHS OF TRIMMED METACARPUS, TIBIA, FEMUR, METATARSUS AND ULNA-RADIUS [a]

Item	Metatarsus	Tibia	Femur	Ulna-radius
Bone weight				
Metacarpus	0.96	0.94	0.89	0.95
Metatarsus		.96	.91	.94
Tibia			.97	.97
Femur				.94
Bone length				
Metacarpus	.97	.96	.80	.88
Metatarsus		.88	.81	.88
Tibia			.96	.97
Femur				.95

[a] All correlations significant at $P<.01$.

stant) between live cannon circumference and ribeye area. Merkel (1958) did find significant partial correlations (carcass weight held constant) between live cannon circumference and percent commercial round and the combined percentage of round, rib, loin, and chuck in beef heifers.

Highly significant correlations were found between the weights and between the lengths of metacarpus, metatarsus, tibia, femur and ulna-radius, as shown in table 2. These high correlations are additional evidence that bones within an animal develop proportionately both in length and weight. This is in agreement with the report of Butler *et al.* (1956).

Simple and partial (chilled carcass weight held constant) correlation coefficients between trimmed bone weights and weights of certain beef cuts and area of ribeye muscle are shown in table 3.

Table 4 gives the simple and partial correlation coefficients between weight: length ratios (which would seem to be a more nearly accurate indicator of bone thickness than bone weight alone) of certain bones and weights of various beef cuts and area of ribeye muscle.

TABLE 3. SIMPLE AND PARTIAL CORRELATION COEFFICIENTS OF TRIMMED BONE WEIGHTS WITH CERTAIN BEEF CUT WEIGHTS AND AREA OF EYE MUSCLE [a]

Item	Trimmed bone weight	
	Metacarpus	Tibia
Sum loin, rib, round and rump	0.63(.64)[b]	0.74(.73)
Sum "retail trimmed" chuck, rib and loin	.69(.66)	.78(.70)
"Retail trimmed" boneless cushion round and rump	.67(.50)	.80(.65)
Area ribeye muscle	.65(.49)	.69(.51)

[a] All correlations significant at $P<.01$.
[b] Partial correlation coefficients (chilled carcass weight held constant).

These correlations indicate that a positive relationship between bone thickness and muscling existed in the 28 steers in this study. It should be kept in mind, however, that only 28 animals representing one breed were studied. Additional studies on bone-muscle relationships are under way at this Station.

TABLE 4. SIMPLE AND PARTIAL CORRELATION COEFFICIENTS OF WEIGHT:LENGTH RATIOS OF METACARPUS AND TIBIA WITH WEIGHTS OF VARIOUS BEEF CUTS AND AREA OF RIBEYE MUSCLE [a]

Item	Weight:length ratio	
	Metacarpus	Tibia
Sum loin, rib, round and rump	0.58(.60)[b]	0.58(.63)
Sum "retail trimmed" chuck, rib and loin	.65(.70)	.66(.70)
"Retail trimmed" boneless cushion round and rump	.63(.50)	.66(.54)
Area ribeye muscle	.61(.46)	.59(.43)

[a] All significant at $P<.01$ except .46 and .43 which are significant at $P<.05$.
[b] Partial correlation coefficients (chilled carcass weight held constant).

Summary

The relationship of bone to muscle in beef carcasses from 28 yearling Hereford steers, fed for 215 days, with an average weight of 919 lb. at slaughter, was investigated.

Weight, length and weight:length ratios were obtained on the metacarpus, metatarsus, tibia, femur, and radius-ulna. Bone weights and bone weight:length ratios were correlated with weights of sum of loin, rib, round and rump and sum of "retail trimmed" chuck, rib, loin, and "retail trimmed" boneless cushion round and rump, and area of ribeye, which were used as indicators of muscling.

The strong positive correlations obtained indicate that bones of an animal develop proportionately in length and weight and suggest that a real association existed between bone thickness and muscling of the cattle studied.

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ACTIVITIES OF DRIED RUMEN MICROORGANISMS *IN VITRO* [1, 2]

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RECENT emphasis on the importance of microorganisms in ruminant nutrition has resulted in the production of a number of commercial products which purportedly stimulate microbial activity by furnishing dried, viable rumen organisms. Several studies have been conducted to determine the effectiveness of feeding these products to ruminants, but, except for their cellulolytic ability, little information is available concerning the activities of these dried microorganisms *in vitro*. Furthermore, the *in vitro* cellulose digestion studies previously reported have usually been made on only one or two different products. The experiments reported below were conducted (1) to test the comparative activities *in vitro* of several commercial dried rumen products in the digestion of cellulose, degradation of carboxymethylcellulose, utilization of non-protein nitrogen, and synthesis of certain B-vitamins, (2) to compare these activities with those of fresh rumen fluid and of rumen material dried in the laboratory under known conditions, and (3) to study the effects of different cultural conditions on the reactivation of dried rumen bacteria.

Experimental

Commercial samples of dried rumen preparations representing five manufacturers were used in the experiments; two companies supplied more than one control lot of their product, and one supplied some experimental batches.

In Vitro Cellulose Digestion Tests. Inocula from the dried rumen preparations were prepared by two procedures. In the first, the desired weight of preparation was added directly to the fermentation flasks and a control was prepared by autoclaving an identically inoculated flask at 121° C. for 15 minutes. Controls so prepared contained the same material as the experimental flasks except for viable microorganisms. The second, and most frequently used method, consisted of adding the desired weight of dried material to 100 ml. of filtered, autoclaved growth medium (Huhtanen *et al.*, 1952), and incubating the mixture anaerobically for 18–24 hours. The insoluble material was removed either by filtration through Reeve Angel No. 812 filter paper, or by centrifugation at 225 × *g.* for 1 minute. A 25-ml. portion of the filtrate (or supernate) was used to inoculate each fermentation flask. Although this procedure may lead to enrichment of certain types of bacteria, the "preincubation" under anaerobic conditions was believed to be beneficial in reactivating any viable, dried, cellulolytic organisms which are notoriously anaerobic. Also, the procedure eliminated the insoluble carrier materials found in commercial dried preparations, and thus prevented their interference in the gravimetric determination of residual cellulose.

Inocula of fresh rumen fluid were prepared from ingesta taken from a fistulated steer. The fluid was strained through cheesecloth and centrifuged at 475 × *g.* for 1 minute to remove vegetable matter and protozoa; 25 ml. were used to inoculate each fermentation flask.

The fermentation procedures for cellulose digestion tests were identical with those used previously (Johnson *et al.*, 1959). Each fermentation bottle contained 800 mg. of cellulose [6] suspended in 50 ml. of double strength medium (Cheng *et al.*, 1955). To this was added 25 ml. of inoculum, prepared as described above from either fresh rumen fluid or from a dried rumen preparation, and 25 ml. of distilled water. After an incubation period of 24 hours at 39° C., residual cellulose was determined by the method of Crampton and Maynard (1938).

Carboxymethylcellulose Hydrolysis. One

[1] Aided by a grant from Vitamins, Inc., Chicago, Illinois.
[2] We are grateful to the following companies for generous supplies of dried rumen preparations: Vitamins, Inc., Chicago, Illinois; B. A. Lucius Co., Fort Worth, Texas; The Cudahy Laboratories, Omaha, Nebraska; Fort Dodge Laboratories, Fort Dodge, Iowa; and Norden Laboratories, Lincoln, Nebraska.
[3] Taken from a thesis submitted by the senior author to the Graduate School of the Agricultural and Mechanical College of Texas in partial fulfillment of the requirements for the degree Master of Science.
[4] Present address, Alcon Laboratories, Forth Worth, Texas.
[5] Department of Biochemistry and Nutrition.
[6] Solfa-floc 40A, Brown Co., Berlin, New Hampshire.

gm. of carboxymethylcellulose (CMC) [7] was dissolved in 100 ml. of single strength medium (Cheng *et al.* 1955), and the solution inoculated by direct addition of the desired weight of dried rumen preparation. Incubation was carried out under CO_2 at 39° C. Alternatively, a few experiments were run with suspensions of bacteria obtained by shaking the dried preparation in water and removing the carrier materials by centrifugation or filtration. This procedure was designed to prevent interference with the viscometric determination of CMC by insoluble matter in the carrier. Hydrolysis of CMC was determined as follows. Immediately after inoculation of the fermentation mixture, and again at the end of the incubation period, samples were withdrawn and the efflux time for each was determined in a Cannon-Fenske viscometer at 40° C. The degree of hydrolysis of CMC was calculated from a standard curve relating efflux time to the concentration of CMC in the basal medium.

Non-Protein Nitrogen Utilization. A 100-ml. quantity of medium (Cheng *et al.* 1955) was supplemented with 0.25 gm. of additional urea and 1 gm. of glucose. The solution was inoculated by direct addition of 0.6 gm. of dried rumen material, and a 10-ml. aliquot was removed immediately for determination of non-protein nitrogen (NPN) initially present. Another sample was taken for NPN determination after the mixture had been incubated anaerobically for 24 hours. Urea nitrogen and ammonia nitrogen were determined by the methods of Pearson and Smith (1943) as modified by Prescott (1953).

Vitamin Synthesis. The medium of Cheng *et al.* (1955) was supplemented with glucose to serve as an energy source. The solutions were inoculated by direct addition of a sample of dried rumen preparation. An aliquot was removed for determination of vitamins initially present and the remainder of the mixture was incubated under CO_2 for 24 hours. The concentration of vitamins present at the end of the incubation period was then determined. Assay procedures were as follows: riboflavin, fluoro-photometric (Najjar, 1941); niacin and vitamin B_{12}, microbiological, U. S. Pharmacopeia XIV, as described by Hawk, Oser, and Summerson (1955).

Lyophilization Procedures. Lyophilization was carried out on homogenized whole rumen ingesta, and on fresh rumen fluid which had been strained through cheesecloth and centrifuged at 475 × *g.* for 1 minute. The samples were shell frozen and placed on the freeze-drying apparatus until dry. In some batches, dry non-fat milk solids and cellulose [6] were added at levels of 2.0 gm. and 1.6 gm. per 20 ml. of rumen fluid, respectively. These additions were made before the preparations were frozen, in order to determine whether they exerted any protective effect on the microorganisms during the drying process (Flosdorf, 1949). Lyophilized samples were stored in stoppered serum bottles at —10° C. until used.

[7] Type 70, Hercules Powder Co., Wilmington, Delaware.

Results and Discussion

In Vitro Cellulose Digestion. In repeated experiments, the commercial dried rumen samples produced little if any cellulose digestion (table 1). Under identical conditions, inocula from fresh rumen liquid consistently digested from 25% to 65% of the added cellulose. Treatments used in preparing inocula produced no improvement. Autoclaved rumen liquid, alfalfa leaf meal extract, valeric acid, and various amino acids reported to stimulate cellulose digestion by inocula prepared from fresh rumen contents (Bentley *et al.*, 1954; Dehority *et al.*, 1957) failed to enhance cellulose digestion by the dried preparations. Lyophilized samples prepared in the laboratory were inactive in cellulose digestion, and no improvement resulted from lyophilizing in the presence of either dry, non-fat milk solids or cellulose.

These results appear to be in general agreement with those of previous investigators.

TABLE 1. DIGESTION OF CELLULOSE BY DRIED RUMEN PREPARATIONS [a]

Commercial preparation used for inoculum	Cellulose digested in trial no.[b]					
	1[c]	2[c]	3[d]	4[e]	5[f]	6[g, h]
	%	%	%	%	%	%
A	2	1[i]	5	0	0	0
B	0	0[h]	2	0	0	..
C	0	0[i]	0	1	0	0
D	0	0[i]	0	0	0	0
E	0	0[i]	0	0	0	0
F	0	0[h]	0	0	0	0

[a] In 55 determinations using inocula prepared from fresh rumen liquid, the mean cellulose digestion value was 47%.
[b] Single determinations unless otherwise indicated.
[c] Dried rumen preparation (DRP), 2 gm.; preincubation period 24 hr.
[d] DRP, 2 gm.; preincubation period 48 hr.
[e] DRP, 3 gm.; preincubation period 60 hr.
[f] DRP, 3 gm.; preincubation period 24 hr.
[g] Inoculum consisted of 2 gm. DRP added directly to fermentation flasks.
[h] Average of three determinations.
[i] Average of four determinations.

TABLE 2. UTILIZATION OF NPN AND SYNTHESIS OF SOME B-VITAMINS BY FRESH RUMEN LIQUID AND DRIED RUMEN PREPARATIONS

Source of inoculum	NPN utilization, mg. per gm. of inoculum [b]	Vitamin synthesis per gm. of inoculum		
		Riboflavin [c] $\mu g. \times 10^4$	Niacin [d] $\mu g.$	Vitamin B_{12} $\mu g.$ [b]
Fresh rumen liquid [a]	36.7	250	624	210
Lyophilized rumen ingesta	1.0	2087 [e]	7	0
Commercial dried preparations:				
A	0.7	32	3	0
B	2.0	10	2	0
C	2.5	14	5	0
D	1.0	22	1	0
E	0.9	13	6	0

[a] Fresh rumen liquid is reported on the basis of activity per ml. rather than activity per gm. Inocula were prepared as described in text.
[b] Average of eight determinations.
[c] Average of two determinations.
[d] Average of four determinations.
[e] Single determination.

Pounden *et al.* (1954) found that a commercial dried preparation gave only 30% as much digestion as fresh rumen juice, and Tucker *et al.* (1956) observed no digestion of filter paper strips incubated for 72 hours with a commercial preparation. Kamstra, Zimmer and Embry (1959) reported cellulose digestion values of less than 10%, and 12.5% for two commercial samples of dried rumen products, and values of less than 10% for lyophilized fresh rumen fluid (30–36 hour fermentation).

NPN Utilization and Vitamin Synthesis. From the data in table 2 it is evident that utilization of NPN and synthesis of three B-vitamins were extremely low by all of the dried commercial and lyophilized preparations; possibly the only exception was the synthesis of riboflavin by lyophilized rumen ingesta. On the other hand, fresh rumen liquid was highly active both in NPN utilization and in vitamin synthesis. Published reports on the utilization of NPN by dried rumen preparations are lacking, although Pounden *et al.* (1954) indicated that an insignificant amount of protein was synthesized under their experimental conditions. The synthesis of B-vitamins *in vitro* by dried preparations has not been reported previously.

Carboxymethylcellulose Hydrolysis. In contrast to their lack of activity toward cellulose, all of the dried preparations degraded CMC, some degrading it to a very great extent (table 3). Fresh rumen liquid digested both cellulose and CMC. Results obtained with the dried preparations, however, indicate that their

TABLE 3. DEGRADATION OF CARBOXYMETHYLCELLULOSE BY DRIED RUMEN PREPARATIONS

Trial no.	Amount of inoculum	Incubation time	CMC degraded by commercial preparation [a]				
			A	C	D	E	F
	gm.	hours	%	%	%	%	%
1	2 [b]	12	79 [d]	..	27 [d]	41	..
1	2 [b]	24	82 [d]	..	46 [d]	64	..
2	2 [b]	18	86	18	28	30	25
3	1.6 [c]	18	76	5	..	28	10 [d]

Trial no.	Amount of inoculum	Incubation time	CMC degraded by sample [e]						
			1	2	3	4	5	6	7
			%	%	%	%	%	%	%
4	1.6 [c]	18	9	35	30	30	36	41	38

[a] Values represent average of two determinations unless otherwise specified.
[b] Inoculated by direct addition of dried rumen preparation (DRP) to fermentation flask.
[c] Inoculum prepared by extracting DRP with water, centrifuging, and discarding precipitate.
[d] Single determination.
[e] Experimental samples kindly supplied by Vitamins, Inc. Details of preparation not specified.

activities toward these two materials represent different specificities. Meiske *et al.* (1958) have demonstrated that the ability of rumen fluid to reduce the viscosity of CMC solutions is not necessarily related to its ability to hydrolyze cellulose. Other investigators have shown that many microorganisms which do not attack cellulose are able to degrade cellulose derivatives (Reese *et al.*, 1950; Levinson and Reese, 1957). The activity of dried rumen preparations toward CMC in our experiments appears to be due to reactivation of organisms capable of hydrolyzing CMC but incapable of hydrolyzing cellulose. In view of these results, CMC appears to be an unsatisfactory substrate for the measurement of true cellulolytic activity. It seems likely that the observed low activities *in vitro* of dried rumen preparations result from decimation of desirable microorganisms during the drying process.

Summary

Samples of commercial dried rumen preparations and lyophilized rumen fluid prepared in the laboratory were compared with fresh rumen liquid with respect to their abilities to digest cellulose, to degrade carboxymethylcellulose (CMC), to utilize urea nitrogen, and to synthesize riboflavin, niacin, and vitamin B_{12}. None of the commercial preparations or laboratory lyophilized preparations showed effective ability to digest cellulose *in vitro* during 24-hour incubation periods. Varying the cultural conditions and adding materials reported to stimulate cellulose digestion by inocula prepared from fresh rumen contents failed to enhance the activity of these preparations. However, all of the dried preparations degraded CMC, thus indicating that organisms attacking this substrate are not necessarily true cellulose digesters. Utilization of urea nitrogen and vitamin synthesis were also much lower by the dried rumen products than by fresh rumen liquor.

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SELECTION AGAINST CRYPTORCHIDISM IN ANGORA GOATS

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IN this paper the term cryptorchidism will be used to describe a condition of either one or two retained testicles. This defect is also commonly called "ridgling," while "toruno" is also used in the Southwest to describe cryptorchidism in sheep and goats. Cryptorchidism is of relatively minor importance to the commercial producer of mohair. The breeder of registered Angora goats experiences a direct monetary loss from the occurrence of cryptorchid male kids, because ranchmen will usually refuse to buy them for breeding purposes, even though most cryptorchids have only one testicle undescended and are fertile. In most flocks they are castrated on the normal side which nearly always results in sterility. They are then used for mohair production, brush control, or for meat. The cryptorchids from flocks of registered Angoras return only a fraction of the amount of money that they would have commanded if they had been normal instead of cryptorchid.

Breeders of Angora goats have long been aware of the problem and of its importance. As an example Gist stated in 1923: "So far as I know, there is no purebred flock of Angora goats in America that is wholly free from this defect; that is, occasionally a ridgling kid will be dropped when only normal or apparently normal sire and dam are mated." Another breeder, Wolf (1923), reported a few breeding observations which pointed toward the inheritance of the cryptorchid defect in Angoras. Lush *et al.* (1930) published results of definite breeding experiments conducted at this Station in the 1920's. Their conclusions were that most, if not all, cases were due to inheritance and that the genes were recessive and relatively few in number. Additional work in the early 30's with the same group of goats by Warwick (1936) confirmed the above conclusions.

Procedure

A flock of registered Angoras was established by the Texas Agricultural Experiment Station in 1917 at its Spur Substation, transferred to the Sonora Substation in 1919 and to the McGregor Substation in 1948. This flock will be designated Flock #1. It was not maintained for the purpose of studying the inheritance of cryptorchidism, yet the selection pressure was radically different during the first 20 years from that of the next 12 years and from the last 11 years. The results should be illuminating as to what may be accomplished toward reduction of the incidence of a defect such as cryptorchidism by increased selection pressure against the defect. Additional evidence for the effectiveness of selection against the defect may be observed from data on Flock #2. This was originally the experimental flock used in the study of cryptorchidism by Lush *et al.* (1930) from 1921 to 1929. It was transferred from Sonora Substation to College Station in 1928 and to McGregor in 1948. The first three kid crops were sired by both cryptorchid and normal bucks. From 1924 to 1930 all the kids were sired by cryptorchids. After 1930 part of the sires were normal. The flock had additions for other experiments in 1930, 1935, 1938 and 1948. One cryptorchid buck, originally used to test susceptibility to stomach worms, lived, so was used for breeding. Two additional cryptorchids were used. Beginning in 1939, only normal sires were used, except in 1943, 1944, and 1945, when these three cryptorchids sired part of the kids. From 1946, no cryptorchid sires were used and the selection pressure was increased. This flock had additions of crossbred goats, goats showing some Angora breeding, goats with no mohair, high grade Angoras and registered Angora females. However, the registered females were bred to bucks of mixed breeding raised in the flock, so the kids were not registered Angoras. In spite of the

[1] Professor, Retired March 1, 1960, Department of Animal Husbandry. Grateful acknowledgment is made of the value of the work of the following persons, each of whom had an important part, at one or more locations, with the breeding records: J. M. Jones, O. L. Carpenter, the late W. H. Dameron, L. J. McCall, R. O. Berry, C. A. Holden, Charles Turner and Eli Gray. Data for the years 1958 and 1959 were supplied through the courtesy of Maurice Shelton.

numerous uses to which the animals were put and the diversity of the additions, it is possible to divide the time periods on the basis of the intensity of selection in each flock, even though there were slight exceptions which are explained.

The classification as to whether a kid is normal or cryptorchid may be made at birth, as was done at College Station and McGregor, but may be made more conveniently at weaning time, as at Sonora.

For the purposes of this paper, the different intensities of selection pressure are defined as follows:

(1) Negative—Deliberate selection for the defect.

(2) None—Selection without reference to the defect, either phenotypic or genotypic.

(3) Slight—Taking special care to avoid cryptorchids when selecting at least part of the sires.

(4) Mild—Use of phenotypically normal sires only, and the culling of none or only a very few of the females genotypically, i.e., *after* they gave birth to a phenotypically defective offspring.

(5) Moderate—Use of phenotypically normal sires only and the culling of all sires and dams genotypically, i.e., *after* defective progeny are born. This does not include culling of earlier progeny.

(6) Rigid—As in (5) plus culling progeny of known carriers.

(7) Very rigid—As in (6) plus culling of other close relatives of known carriers.

Results and Discussion

During the first period of 20 years of Flock No. 1 (table 1 and figure 1) the selection pressure against the defect was "mild". Little or no attention was given to progeny test information in regard to cryptorchidism. The incidence of cryptorchidism fluctuated from year to year from 0 to 6.8% cryptorchids. This may be slightly higher than average for stud flock goats, yet during this period many of the sires used were bred by prominent breeders. These sires included one stud imported from South Africa; many of them, including the imported buck, sired one or more cryptorchids. Some of the sires were continued in service for several years after siring cryptorchids. In one case a buck that sired a cryptorchid in his first kid crop was used for 9 more years.

This was followed in Flock No. 1 by 12 years of moderate selection pressure, which included culling genotypically. As before, no cryptorchids were used as sires. Every doe that gave birth to even one cryptorchid was culled from the flock, but her previous progeny were not culled. Most of the sires were also culled if they sired even one cryptorchid. There were four exceptions to this. These four bucks sired 29 male kids after they were known to have sired cryptorchid offspring. During the years these four bucks were used after it was known that they were carriers, 181 male kids were sired by other bucks. Thus, even though the culling of known carrier bucks was not 100%, the selection pressure against cryptorchidism could well be considered moderate. During this period of 12 years, 789 male kids were born, of which

TABLE 1. REGISTERED ANGORA GOAT FLOCK. FLOCK NO. 1

Year	Number males	Number cryptorchids	Percent
1917	8	1	12.5
1918	5	0	0
1919	12	1	8.3
1920	33	1	3.0
1921	36	1	2.8
1922	28	4	14.3
1923	44	2	4.5
1924	59	1	1.7
1925	58	0	0
1926	83	11	13.3
1927	67	5	7.5
1928	92	3	3.3
1929	78	1	1.3
1930	65	4	6.2
1931	134	7	5.2
1932	85	9	10.6
1933	96	5	5.2
1934	56	7	12.5
1935	56	7	12.5
1936	73	9	12.3
1937	65	3	4.6
1938	59	2	3.4
1939	75	0	0
1940	83	4	4.8
1941	54	0	0
1942	67	4	6.0
1943	65	3	4.6
1944	70	3	4.3
1945	75	1	1.3
1946	60	2	3.3
1947	73	0	0
1948	52	1	1.9
1949	3	0	0
1950	23	0	0
1951	23	0	0
1952	36	0	0
1953	20	0	0
1954	15	0	0
1955	20	1	5.0
1956	20	0	0
1957	19	0	0
1958	24	0	0
1959	24	0	0

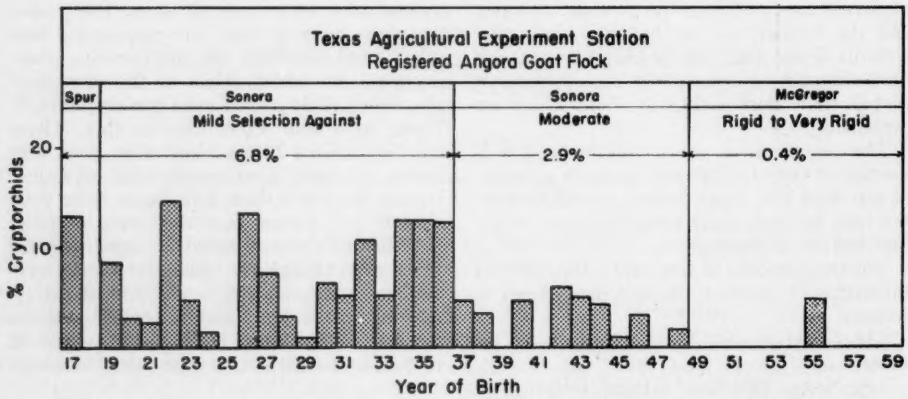

Figure 1. Changes in occurrence of cryptorchidism in Angora Goats, according to degree of selection pressure against the defect.

TABLE 2. FLOCK NO. 2

Year	Number males	Number cryptorchids	Percent
1921	105	6	5.7
1922	93	5	5.4
1923	59	6	10.2
1924	35	4	11.4
1925	105	25	23.8
1926	45	18	40.0
1927	66	21	31.8
1928	64	19	29.7
1929	48	21	43.8
1930	12	6	50.0
1931	17	6	35.3
1932	19	12	63.2
1933	12	6	50.0
1934	7	4	57.1
1935	27	10	37.0
1936	10	4	40.0
1937	18	6	33.0
1938	29	4	13.8
1939	31	4	12.9
1940	17	1	5.9
1941	26	4	15.4
1942	23	1	4.3
1943	28	4	14.3
1944	65	3	4.6
1945	33	2	6.1
1946	34	1	2.9
1947	31	0	0
1948	37	1	2.7
1949	64	1	1.6
1950	91	1	1.1
1951	79	0	0
1952	76	1	1.3
1953	106	1	0.9
1954	97	1	1.0
1955	26	0	0
1956	56	1	1.8
1957	68	0	0
1958	61	0	0
1959	64	0	0

23, or 2.9%, were cryptorchids. Even though there were a few exceptions during the period of culling known carrier sires, the incidence of cryptorchidism dropped from 6.8% to 2.9%; and the highest in any particular year was 6.0%, which is lower than the average of the preceding 20-year period.

During the last 11 years (in Flock No. 1) it was possible to use rigid to very rigid culling against the genes for cryptorchidism. This included in addition to the moderate culling practiced in the previous 12 years period, the culling of some previous progeny of known carriers, and some other relatives of known carriers. Only one of the 227 males born was a cryptorchid. This is 0.4% in contrast to 6.8% in the period of mild selection pressure and 2.9% during moderate selection pressure.

Flock No. 2 (table 2 and figure 2) was originally used in the study of the inheritance of cryptorchidism when cryptorchid sires were used. This negative selection resulted in a very high frequency of does carrying one or more of the genes for cryptorchidism. Additions of some radically different breeding were made to this flock, yet cryptorchids were produced from each group added. The first three kid crops (1921, 1922, and 1923) included kids sired by normal bucks as well as cryptorchids. Of the 257 male kids, 17, or 6.6%, were cryptorchids. The 1924 to 1930 kids were all sired by cryptorchids. Of the 375 male kids, 114, or 30.4%, were cryptorchids. During the next 4 years most of the kids were sired by cryptorchids, but one normal sire was used each year. Of the 55 male kids

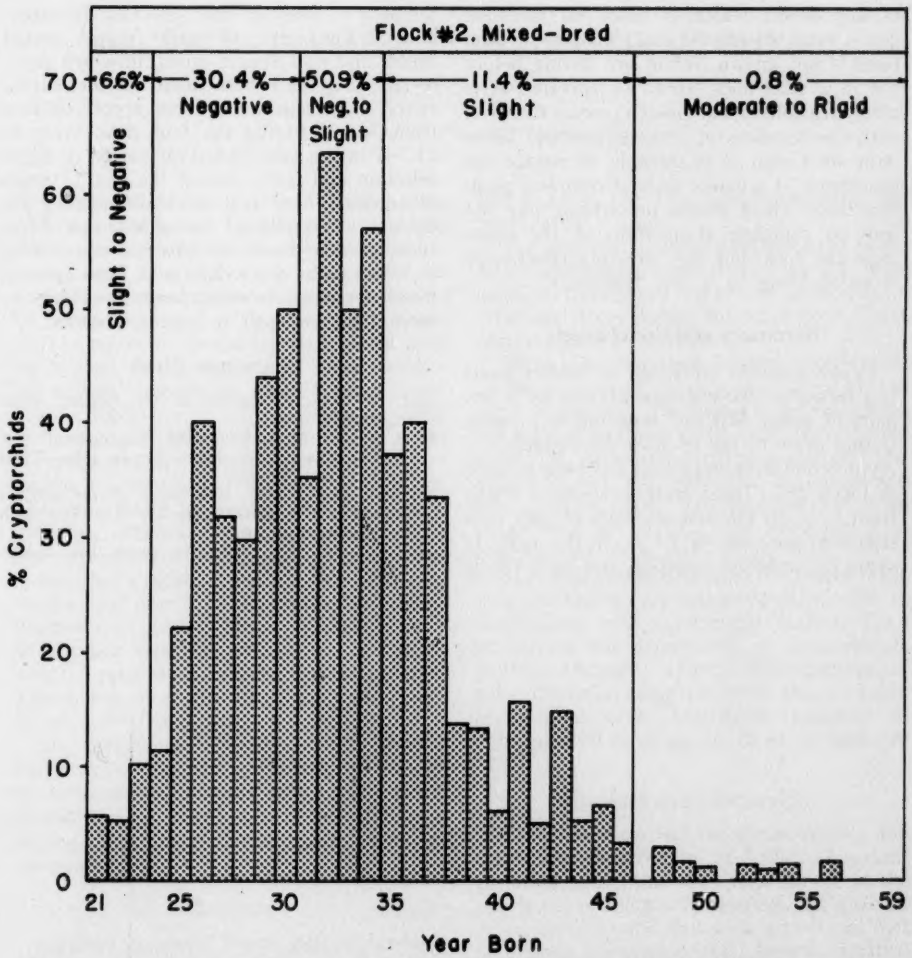

Figure 2. Changes in occurrence of cryptorchidism in a flock with grade Angora, crossbred, and mixed bred goats according to the degree of selection pressure against the defect.

born, 28, or 50.9%, were cryptorchids. From 1935 to 1946 the flock had additions for other experiments, but the use of cryptorchid sires was continued through 1938. Three more cryptorchid sires were used in the years 1943, 1944, and 1945. Even though some cryptorchid sires were used, the selection pressure was changing from slight toward moderate. The 341 male kids born included 40 cryptorchids, which is 11.7%. From 1947 to 1959, inclusive, with moderate to rigid selection pressure the incidence was 7 of 856 male kids, which is 0.8%.

The recommendations for the reduction of the incidence of this defect, published by Lush *et al.* (1930) was somewhat cautious. In light of the data to that date this caution was well justified. The recommendations of Lush *et al.* in 1930 are quite similar to many present day recommendations (including the caution) for other recessive defects. Much of the caution against too rigid genotypic selection pressure can now be removed from the recommendations.

These results should be a valuable guide to selection aimed at reducing the occurrence

of any defect which is based on recessive genes, even though the exact number of gene pairs is not known. While sire testing before use in a stud flock would be effective, it is rarely feasible to test enough prospective sires with the numbers of progeny needed. These data show that it is possible to reduce the occurrence of a defect without complete progeny tests. These simple procedures may not lead to complete elimination of the genes from the flock, but they are very effective in reducing them to a low frequency.

Summary and Conclusions

Cryptorchidism (ridgling) of Angora goats is a recessive character conditioned by a few pairs of genes. Without resorting to progeny testing prior to use of sires the incidence of cryptorchidism in male kids has been reduced in Flock #1 (Texas Station registered flock) from 6.8% in the first 20 years of only mild selection pressure to 2.9% in the next 12 years of moderate selection and to 0.4% in the next 11 years of rigid selection. In experimental Flock #2, of grade Angora, mixed breed, and non-Angora goats, in which cryptochid sires had been used in the earlier years, slight selection reduced cryptorchidism from 50.9% during the four peak years to 11.7% in the next eight-year period of slight selection and 0.8% during the last 13 years of moderate to rigid selection against the defect. It is concluded that simple procedures such as these would be effective in reducing to a low point other defects in farm animals which are recessive even though there may be more than one pair of recessive genes.

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PLACENTAL TRANSFER OF MANGANESE IN SWINE [1, 2]

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MANGANESE has been found to be present in the new-born pig (Johnson, 1943 and 1944), and thus is known to cross the placenta of the sow. The manganese requirement for normal fetal development in swine appears to be between 0.5 and 6.0 ppm. since Johnson (1943) reported normal pigs born from sows maintained on semi-synthetic diets containing 6 ppm. manganese, while Plumlee *et al.* (1956) found resorption of fetuses or birth of small, weak pigs which could neither stand nor walk properly, from sows on semi-purified rations containing 0.5 ppm. of manganese. The requirement for normal estrus and fertility is less conclusive. Grummer *et al.* (1950) observed lowered fertility and irregular estrus in sows fed a ration of natural foodstuffs containing 12 ppm. of manganese. However, Plumlee *et al.* (1956) found complete absence or irregular estrus in gilts grown on rations with 0.5 ppm. of manganese but no apparent disturbance of estrus when the ration contained 1.5 or 3.5 ppm.

The purpose of this study was to observe the fetal development and placental transfer of manganese at various stages of gestation in sows fed rations of natural foodstuffs containing high and relatively low levels of manganese.

Methods

Eighteen purebred Duroc gilts of breeding age were divided into two lots according to weight and inheritance, and housed in pens with concrete floors. The experimental rations used and methods of handling the gilts have been described in detail in a previous paper (Newland *et al.*, 1960). The animals received the experimental rations four weeks prior to breeding and during the gestation period until sacrifice. The gilts in Lot 1 received a ration containing approximately 100 ppm. manganese and those in Lot II a ration containing approximately 6 ppm.

The gilts which received injections of radioactive manganese, one from each lot, were kept in metabolism racks for collection of urine and feces during the three-hour, injection-to-sacrifice interval.

Three gilts from each lot were sacrificed at 60 days, three at 80 days and three at 110 days of gestation. They were killed by cutting the jugular vein and aortic arch. Immediately afterward the entire uterus with contents was removed, and the respective maternal and fetal samples taken. The samples were dried in an oven at 100° C., and wet-ashed with concentrated nitric and sulfuric acid.

Radiomanganese determinations were made with immersion-type Geiger-Mueller tubes in conjunction with commercial scalers. Total manganese was determined by the periodate method (Sandell, 1950), determination of color intensity being made on the Beckman Spectrophotometer. Statistical treatment of data followed methods described by Snedecor (1950).

Results and Discussion

Table 1 summarizes the mean weights and total manganese content of fetuses of various gestation ages from sows maintained on the two levels of dietary manganese. An analysis of variance showed that with a maternal diet of 6 ppm. manganese, fetal growth equalled that obtained with a diet of 100 ppm. The level of manganese in the maternal diet had a significant influence on placental transfer to the fetuses. The average concentration of manganese in fetuses from sows fed the high level of manganese was 5.33 ppm. on the dry basis, as compared to 1.35 ppm. in fetuses from sows fed the low level (table 1). Skinner *et al.* (1931) and Orent and McCollum (1932) found this relationship in rats and Plumlee (1956) in pigs. However, Bentley and Phillips (1951) reported that calves of cows on rations containing less than 10 ppm. of manganese were born with weak front legs although manganese content of fetal blood and liver was not significantly lowered.

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TABLE 1. AVERAGE FETAL WEIGHT (FRESH) AND TOTAL MANGANESE CONCENTRATION IN FETUSES OF DIFFERENT AGES FROM SOWS FED HIGH AND LOW LEVELS OF MANGANESE

Fetuses from Lot I sows, 100 ppm. Mn in diet					Fetuses from Lot II sows, 6 ppm. Mn in diet			
		Av. total Mn					Av. total Mn	
No. fetuses analyzed	Av. wt., gm.	Fresh, ppm.	Dry, ppm.		No. fetuses analyzed	Av. wt., gm.	Fresh, ppm.	Dry, ppm.
13	130 (21)[a]	0.818	7.47	60-day fetuses	14	119 (38)	0.141	1.33
9	396 (28)	0.484	4.47	80-day fetuses	13	427 (21)	0.196	1.75
6	1171 (31)	0.690	4.05	110-day fetuses	6	945 (24)	0.192	0.98
Av.		0.664	5.33**				0.176	1.35

[a] Total number fetuses in litters from 3 sows is shown in parenthesis.
** Highly significant over Lot II ($P<0.01$).

The concentration of total manganese in the blood and certain maternal organs is presented in table 2. Of the organs studied, the liver and ovaries were highest in manganese. There was a slight tendency for higher concentration of manganese in the organs (except ovaries) of sows fed the higher manganese ration. A similar tendency was noted by Grummer *et al.* (1950) in pigs fed high-manganese diets.

The ovaries of the sows receiving the low level of manganese were slightly higher in manganese than those from sows on the high level. Each of the gilts started on the experiment exhibited normal estrus and conceived. Bentley and Phillips (1951) found 0.85 and 2.00 ppm. of manganese in the ovaries of cows maintained on rations containing 10 and 30 ppm. of manganese, respectively, and postulated a relationship between manganese deficiency and low fertility in cattle.

Because of the low concentration, it was necessary to pool several samples for the determination of blood manganese. The sows in Lot I, on high dietary manganese, had an average of 0.450 mcg. of manganese per ml. of blood serum, as compared to 0.275 mcg. per ml. in the serum of sows on low manganese. Thus, manganese in the maternal serum varied directly, but apparently not proportionally, with the dietary level of the element.

TABLE 2. TOTAL MANGANESE IN MATERNAL SAMPLES FROM SOWS ON DIFFERENT LEVELS OF MANGANESE

	Lot I (High Mn)		Lot II (Low Mn)	
Source	No. samples analyzed	Total manganese (dry)	No. samples analyzed	Total manganese (dry)
Liver	9	7.65 ppm.	7	6.42 ppm.
Heart	9	4.28 ppm.	8	3.49 ppm.
Ovaries	8	7.14 ppm.	7	8.71 ppm.
Thyroid	7	2.40 ppm.	5	2.24 ppm.
Blood serum	9	0.450 μg/ml.	7	0.275 μg/ml.
Blood cells	7	0.240 μg/ml.	7	0.179 μg/ml.

In order to obtain information on the rate of placental transfer of manganese, one sow from each lot was injected intraperitoneally with 480 microcuries of radiomanganese[4] ($MnCl_2$ in HCl) three hours prior to sacrifice. Maternal blood samples assayed for radioactivity showed only background counts and thus it was not possible to determine the quantity of total manganese transferred to the fetuses per day.

Although the data are limited, results in table 3 indicate that the level of manganese in the maternal diet had no apparent effect on the rate of placental transfer of $Mn^{52, 54}$ during the three-hour experimental period. No radiomanganese activity could be detected in the fetal livers, hearts, and femurs. It should be pointed out that Newland *et al.* (1960) found significant amounts of P^{32} transferred to fetal livers, hearts, and femurs in swine during a three-hour period.

Summary

Sows maintained on rations of natural foodstuffs containing 6 and 100 ppm. manganese produced fetuses of essentially the same weight. The concentration of manganese in the maternal serum was related to the level in the ration. Furthermore, on the 60th, 80th and 110th day of gestation the concentration of manganese was significantly higher in the fetuses from sows on the higher level of dietary manganese. Radiomanganese was transferred in detectable amounts to the 110-day pig fetus during a three-hour period.

[4] Obtained from the Oak Ridge National Laboratories under the authorization of the Atomic Energy Commission.

TABLE 3. PLACENTAL TRANSFER OF $Mn^{52, 54}$ TO THE 110-DAY FETAL PIG [a]

	Mn in maternal diet, ppm.	Fetal wt. (dry) gm.	Fetal absorption of $Mn^{52, 54}$ (% dose)		
			Total	Per gm. (% dose x 10^3, dry basis)	Per hr./gm.
Lot I	100	152.7	0.4438	2.91	0.97
		250.2	0.1569	0.63	0.21
Lot II	6	223.0	0.6604	2.97	0.99
		241.0	..... [b]		

[a] Findings reported on two fetuses from each sow.
[b] Activity not significantly above background.

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HEMOGLOBIN AND BLOOD CELLS OF GROWING BEEF CATTLE [1]

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STUDIES were undertaken to provide information on changes in hemoglobin and blood cells associated with increases in body weight of presumably normal animals. No attempt has been made to cover all areas of hematology, and only those references pertinent to the present study are presented. The most helpful source of information on blood constituents has been edited by Albritton (1952).

Hemoglobin. The average amount of hemoglobin in the blood of normal beef and dairy cattle of various ages has been reported by various workers to vary from 10.9 to 13.2 gm. per 100 ml. of blood (McCay, 1931; Dukes, 1955; MacDonald *et al.*, 1956; Williams, 1955; Arthaud *et al.*, 1959; Rusoff and Piercy, 1946; Long *et al.*, 1952; Alexander *et al.*, 1959). Rusoff *et al.* (1954) working with Jerseys, Guernseys and Holsteins and Long *et al.* (1952) working with Shorthorn, Angus and Herefords found no breed differences in hemoglobin content of blood; whereas Alexander *et al.* (1959) and MacDonald *et al.* (1956) found that Angus males were higher in hemoglobin than Hereford males. MacDonald *et al.* (1956) and Alexander *et al.* (1959) observed higher hemoglobin content in blood of Hereford and Angus calves at 800 lb. than at 500 lb. body weight.

Red Blood Cells. A range of 4.9 to 9.98 million red blood cells per cu. mm. of blood has been reported for dairy cattle (Coffin, 1953; Dukes, 1955; Ferguson *et al.*, 1945; Greatorex, 1954; Reid *et al.*, 1948; Rusoff *et al.*, 1954) and 4.5 to 9.8 million per cu. mm. of blood for beef cattle (Arthaud *et al.*, 1959; Long *et al.*, 1952; Alexander *et al.*, 1959). No breed differences in red blood cell number have been noted in the literature.

A range in average hematocrit values in dairy cattle from 28 to 50% has been reported (Albritton, 1952; Holman, 1952; Greatorex, 1954) and in beef cattle from 31 to 48% (Arthaud *et al.*, 1959; Alexander *et al.*, 1959). A breed difference in dairy cattle was noted in which Holsteins with 39.5% were low, Jerseys with 42.3% were intermediate and Guernseys with 46% were high (Rusoff *et al.*, 1954). Alexander *et al.* (1959) observed an increase in average hematocrit value from 40.3 to 44.0 as Hereford and Angus calves grew from 500 to 800 lb. body weight.

Mean corpuscular volume in dairy and beef cattle varied from 40.0 to 60.7 cubic microns as reported by Coffin (1953), Albritton (1952), Greatorex (1954) and Alexander *et al.* (1959). Alexander *et al.* (1959) observed an increase of mean corpuscular volume for Hereford and Angus calves from 52.6 cubic microns at 500 lb. body weight, to 53.7 cubic microns at 800 lb. body weight.

An increase in mean corpuscular hemoglobin from 15.9×10^{-12}gm. at 500 lb. to 16.1×10^{-12}gm. at 800 lb. body weight was reported by Alexander *et al.* (1959), whereas mean corpuscular hemoglobin concentration was observed to be 30.3% at both weights. Averages of 32.0 and 37.2% for mean corpuscular hemoglobin concentration were observed by Coffin (1953) and Alexander *et al.* (1959).

White Blood Cells. A range from 8,411 to 10,268 leucocytes per cu. mm. of blood has been reported (Brody *et al.*, 1949; Ferguson *et al.*, 1945; Greatorex, 1954; Rusoff and Piercy, 1946). A breed difference was noted by Rusoff *et al.* (1954) in dairy cattle and Alexander *et al.* (1959) in beef cattle. Alexander *et al.* (1959) reported a decrease in leucocyte count as cattle increased from 500 to 800 lb. body weight.

Greatorex (1954) reported values for different types of leucocytes in dairy cattle from birth to one year of age. Neutrophils were found to decrease from 45 to 28, eosinophils increased from 1.1 to 3.0 and lymphocytes increased from 31 to 67 per cu. mm. There were no consistent quantitative changes in monocytes, and basophils were consistently absent.

Methods and Procedures

Data were taken from 45 beef calves maintained at the Oregon Agricultural Experiment Station at Corvallis, Oregon, in 1958. Two breeds, 32 Herefords and 13 Angus, are rep-

[1] This study was conducted in cooperation with the Agricultural Research Service, U. S. Department of Agriculture, and State Experiment Stations under Western Regional Project W-1 on Beef Cattle Breeding Research and W-46 on Effects of Environmental Stress on Range Cattle and Sheep Production. Technical Paper 1320. Oregon Agricultural Experiment Station.

resented, the Hereford calves being composed of three inbred lines, Lionheart, Prince and David; and the Angus calves represented by only one line.

Management. The calves were born in the spring of 1958 and were maintained on irrigated pastures during the summer months until they reached 425 lb. body weight at which time they were placed under experimental conditions, or until October 29, 1958, at which time the lighter calves were placed under experimental conditions. Thus, all calves were under standard feed-test conditions for a gain of at least 75 lb. before the first blood samples were taken.

The calves were grouped according to sex into pens of six animals. From the first feeding period until the end of the test period, the calves were tied by neck chains at individual feeding stalls twice a day. Feeding periods lasted approximately 3 to 5 hours and were maintained as uniformly as possible. Calves had access to automatic water fountains at all times. The test period started when calves attained 500 lb. body weight and ended at 800 lb. body weight.

The management procedure used and recommended by Dahmen and Bogart (1952) was followed, and the calves were fed a completely pelleted ration composed of two parts chopped alfalfa and one part of concentrate. The ration is described in detail by Nelms *et al.* (1953). All calves were in the same barn and had the same ration throughout the experiment.

Blood Collection. Four samples of jugular blood were collected from each calf, one each at 500, 600, 700, and 800 lb. body weight. Calves were routinely weighed each week and blood samples were taken at the time the animals were nearest to the four weights listed above. All samples were taken at the same time of day just following their morning feeding. Either 13 or 15 gauge bleeding needles were used. Samples of 10 ml. were collected into oxalated tubes to prevent coagulation. The technique was that recommended by Washko (1948), which called for drying oxalate in an oven to prevent disturbing the volume composition of the blood. All blood samples were collected between 9:00 and 10:00 a.m., and examined immediately.

Hematological Study. Observations were made on hemoglobin, hematocrit, total number of red blood cells, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration; also on the number of leucocytes, neutrophils, eosinophils, basophils, lymphocytes and monocytes.

The method for hemoglobin determination has been described in detail by Oldfield (1952). The method for counting red and white blood cells was described by Coffin (1953) and mean corpuscular volume, hemoglobin and hemoglobin concentration were calculated according to Wintrobe (1951). A blood smear for differential white cell count was prepared with Wright's stain as described by Osgood (1940).

Statistical Analysis. Means and tests of differences between the means were carried out according to statistical techniques outlined by Li (1957). Since there were few instances of significant sex, line and breed effects at each of the body weights, the data were analyzed without removing these effects. The 5% level of probability was accepted in tests of significance.

Results

Red Blood Cell and Hemoglobin Changes Associated with Increases in Body Weight. Hemoglobin per 100 ml. blood increased steadily from 11.8 gm. at 500 lb. body weight to 12.4 gm. at 800 lb. body weight. The amount of hemoglobin per red cell increased from 14.9 to 17.1 micromicrograms and the concentration of hemoglobin in the red cells increased from 31.6 to 38.1% with changes in body weight from 500 to 800 lb. (figure 1).

The number of red blood cells consistently decreased from 8.1 million per cu. mm. of blood at 500 lb. to 7.4 million per cu. mm. at 800 lb. body weight. The size of red blood cells showed a decrease but this change was not consistent.

There was a decrease in hematocrit from 37.6 volume % at 500 lb. to 33.3% at 800 lb. body weight. (figure 1).

The data were analysed by use of the t-test and Duncan's multiple range test (Li, 1957) for comparing values obtained on a trait at one weight with values obtained on the same trait at each of the other weights. The increase in hemoglobin whether determined per 100 ml. of blood, micromicrogram per cell or concentration per cell as the calves grew from 500 to 800 lb. body weight was always statistically significant. Thus, consistent increases in hemoglobin exist because of the marked increase in concentration of hemoglobin within the red blood cells. The hematocrit value was substantially greater at 500 lb. body weight than at 600, 700, and 800 lb., but the differences among the values

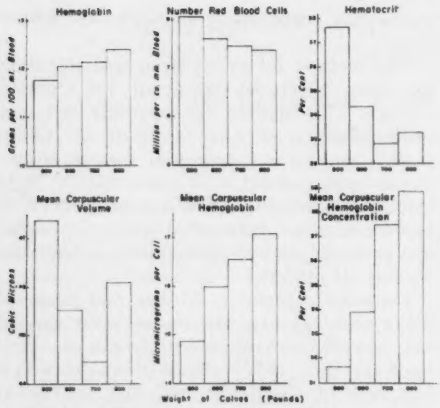

Figure 1. Changes in red blood cells and hemoglobin associated with increases in body weight of beef calves.

obtained at these latter three weights lacked significance. No differences were found among the values obtained for mean corpuscular volume at the various weights; standard deviations were large.

Changes in White Blood Cells Associated with Increases in Body Weight. The number of white blood cells, lymphocytes and monocytes decreased consistently as calves grew from 500 to 800 lb. body weight. The number of white blood cells decreased from 10.0 thousand at 500 lb. to 8.9 thousand per cu. mm. of blood at 800 lb. body weight; lymphocyte number decreased from 6760 to 5970 and monocytes decreased from 494 to 272. (figure 2).

Statistical analyses revealed consistent decreases in total leucocyte, lymphocyte and monocyte counts. There were no differences in values obtained for eosinophils, basophils and neutrophils at the four body weights. It may be of interest to note, however, that no basophils were observed at 500 lb. body weight while a few were present in blood from calves at each of the other body weights.

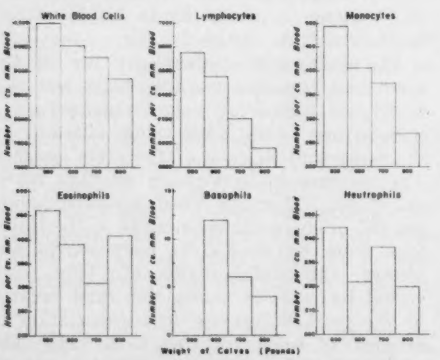

Figure 2. Changes in numbers of white blood cells associated with increases in body weight of beef calves.

Discussion

In any study with growing animals one either relates his observations to weight, age or time. However, it must be recognized that if observations are taken at given body weights, times of observations and ages of calves vary. Since all three of these; time, age and weight are confounded, one recognizes that statements regarding the association of blood physiological changes with changes in body weight must have the confounding error involved with associated variations in age and in time. Weight was used in the present study because it was felt that physiological changes should bear a closer relationship to weight than to age or time.

This study was concerned with changes in blood characteristics associated with increases in body weight of growing beef cattle. It has been shown that consistent changes occur in the blood as animals increase in body weight from 500 to 800 lb. Number and size (as indicated by mean corpuscular volume) of red blood cells decreased while concentration of hemoglobin in whole blood, amount of hemoglobin per red cell (as indicated by mean corpuscular hemoglobin), and concentrations of hemoglobin in individual red blood cells increased with increases in body weight. This would indicate that the fewer and smaller red blood cells in the larger calves have the same or a greater efficiency of each red blood cell in this important function. If fewer and smaller red blood cells can do the same work in larger calves as more and larger red blood cells in smaller calves, it suggests a change in the proportion of cellular-to-plasma components as animals grow. This appears to be the case as indicated by a decrease in hematocrit or volume of packed cells as animals grow from 500 to 800 lb. body weight. Whether the relative increase in blood plasma with increases in calf size would permit blood to carry greater quantities of soluble nutrients is not known. However, the blood and chemical studies by Williams (1955) have shown increases in amino acid nitrogen and urea concentration as calves increase in size.

The results of the present study were not in complete agreement with previous studies re-

ported by Alexander *et al.* (1959), who observed an increase in total number of red blood cells and hematocrit values as calves grew from 500 to 800 lb. body weight. These authors also recorded a constant red cell size and concentration of hemoglobin per red cell as the calves increased from 500 to 800 lb. body weight. This suggests that from time to time environmental factors may alter the size and number of the red blood cells. Arthaud *et al.* (1959) reported both an increase in hemoglobin and in total number of red blood cells as Hereford, Shorthorn and Angus calves grew from 7 months old to 9 months old. Greatorex (1954) reported a decrease in hemoglobin content when calves grew older but no marked difference was observed in total number of red blood cells.

The total number of white blood cells, lymphocytes and monocytes decreased with increases in body weight from 500 to 800 lb., whereas the number of neutrophils, eosinophils and basophils were variable from weight to weight. The present investigation is in agreement with previous work reported by Alexander *et al.* (1959) who also observed a decrease in total number of white blood cells, and in number of lymphocytes and monocytes as Hereford and Angus calves grew from 500 to 800 lb. body weight. Greatorex (1954) has reported a decrease in the number of lymphocytes as calves grew from birth to one year of age and observed the number of monocytes, neutrophils and eosinophils to be variable from age to age while basophils were absent.

Summary

The amount of hemoglobin per unit volume of the blood, hemoglobin content of individual red blood cells, and the concentration of hemoglobin in each red blood cell increased in beef calves (Hereford and Angus) with increases in body weight from 500 to 800 lb. The number of red blood cells decreased with increased body weight.

The number of white blood cells, lymphocytes and monocytes decreased with increases in body weight while the number of neutrophils, eosinophils and basophils were variable from weight to weight.

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ESTRUS, OVULATION AND FERTILITY IN GILTS SUBSEQUENT TO THE ORAL ADMINISTRATION OF 6-METHYL-17-ACETOXYPROGESTERONE [1]

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PREVIOUS communications from this laboratory (Nellor, 1959, 1960) reported that the oral administration of 6-methyl-17-acetoxyprogesterone resulted in accurate control of follicular growth and estrus in gilts. A range of 0.8 to 1.6 mg. of 6-methyl-17-acetoxyprogesterone per pound body weight daily, by twice-a-day feeding shortly before the normal ration, was capable of inhibiting follicular growth and estrous activity during the 25-day treatment period. Estrus and ovulation occurred 4 to 5 days after the end of treatment.

The purpose of the present study was to determine whether follicular growth and estrus could be controlled by the oral administration of 6-methyl-17-acetoxyprogesterone under practical management conditions, that is, when the compound was added to the normal ration, the amount being based on the average body weight and average feed consumption of a group of gilts fed together. Also to determine whether the dosage of the compound and duration of treatment could be less than that utilized in earlier studies, i.e., less than 1.6 mg. per lb. body weight daily for 25 days.

Materials and Methods

One hundred and forty-nine purebred and crossbred gilts, weighing 190 to 310 lb., were used in the study. The gilts were from 6.5 to 8 months old when placed on experiment and were considered sexually mature. No attempt was made to ascertain the stage of the estrous cycle when progestational treatment started.

Forty-one control gilts were bred at the first estrus while on experiment; 26 were autopsied 35 to 45 days following service and 13 gilts carried to term. Two control gilts that remained open after several breedings were removed from the experiment. One hundred and eight gilts were divided at random into groups, each of 7 to 18, and fed rations containing 6-methyl-17-acetoxyprogesterone.[4] The gilts were housed and fed outside throughout the study.

A standard gestation ration (1/3 corn, 2/3 oats and 10% protein supplement) was full-fed before and after the treatment period. The treated rations were prepared by adding 6-methyl-17-acetoxyprogesterone to the protein supplement and this in turn was added to the corn and oats, the entire ration for each trial being prepared at one mixing.

In order to duplicate practical management conditions, the average body weight of the gilts and the average amount of ration offered were used in each trial to calculate the dosage of 6-methyl-17-acetoxyprogesterone to be added to the ration. The dosages referred to in this report, 0.4 to 1.6 mg. per pound body weight daily, therefore, are the "average dosage(s) fed (ADF)" per gilt in any particular trial. The amount of ration fed, based on the average feed consumption of the groups, varied from 3 to 7 lb. per gilt daily, by twice-a-day feeding.

The gilts in the first four trials were checked with boars daily during the treatment period. All of the gilts were checked with boars at least twice daily after the end of progestational treatment to estrus or autopsy. Some of the gilts were bred at the first estrus following the end of treatment and others were bred for the first time at the second estrus following the end of treatment. The majority of the gilts were bred again if recycling occurred.

Gilts coming into estrus 48 to 72, 72 to 96, or 96 to 120 hours after the last feeding of 6-methyl-17-acetoxyprogesterone are reported as coming into estrus 3, 4 and 5 days after the end of treatment, respectively.

The experimental design of the 10 trials is tabulated in table 1.

[1] This study was supported, in part, by The Upjohn Company, Kalamazoo, Michigan. The authors wish to express appreciation to J. B. Stafford and E. C. Miller, Department of Animal Husbandry, for obtaining the gilts and to G. H. Conner, Department of Surgery and Medicine, for the preparation of vasectomized boars. Submitted with the approval of the Michigan Agricultural Experiment Station, June 6, 1960 as Journal Article 2637.

[2] Endocrine Research Unit—Departments of Animal Husbandry and of Physiology and Pharmacology.

[3] Department of Animal Husbandry.

[4] Provera, registered trade name of The Upjohn Company, Kalamazoo, Michigan.

TABLE 1. EXPERIMENTAL DESIGN

Trial	Number of gilts	Av. body weight (lb.)	Progestational treatment[a]	Feed consumption[b] (lb.)	Treatment duration (days)
1	18	217	1.6	5	15–27
2 (Group 1)	7	252	1.6	5	19[d]
(Group 2)	8	252	1.6–.8[c]	5	19[e]
3	10	246	0.6	3	11 or 15[f]
4	10	229	.4	3	12 or 15[g]
5	9	248	.5	3	15
6	9	266	.5	4	15
7	9	258	.5	4	15
8	9	290	.5	4	15
9	9	253	.5	7	7
10	10	276	.5	7	15
	41	234	Control gilts[h]		

[a] Average amount of 6-methyl-17-acetoxyprogesterone per lb. body weight daily, fed in the normal ration by twice-a-day feeding, based on the average body weight and average feed consumption of the group (ADF).
[b] Number of pounds of ration consumed per gilt daily, based on the average feed consumption of the group.
[c] 1.6 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily for 14 days and 0.8 mg. from the 15th day of treatment to the end of treatment 4 days later.
[d] Received 1000 I.U. of equine gonadotropin, subcutaneously, 24 hours after the end of progestational treatment.
[e] One gilt autopsied on the 15th day of treatment and three gilts received 1000 I.U. of equine gonadotropin, subcutaneously, 24 hours after the end of progestational treatment.
[f] Five gilts autopsied on the 11th day of treatment and five gilts continued on treatment for a total of 15 days.
[g] Five gilts autopsied on the 12th day of treatment and five gilts continued on treatment for a total of 15 days.
[h] Autopsied at 35 to 45 days of pregnancy or carried to term.

Three gilts from Trial 1 were autopsied on the 15th day of progestational treatment in order to ascertain the degree of follicular inhibition induced by the treatment. Fifteen gilts remained on treatment 27 days. Gilts coming into estrus 5 to 10 days after the end of treatment were bred and autopsied 35 days from service. The remaining gilts were autopsied 10 to 40 days after the end of treatment.

One gilt was autopsied from Trial 2 on the 15th day of treatment for ovarian examination and seven gilts of Group 1, Trial 2, received 1000 I.U. of equine gonadotropin[5] 24 hours after the end of a 19-day progestational treatment period. The progestational dosage was reduced from 1.6 to 0.8 mg. per lb. body weight daily, for the seven gilts in Group 2, Trial 2, from the 15th to the 19th day of treatment and three gilts received 1000 I.U. of equine gonadotropin 24 hours after the end of progestational treatment. The gilts in Trial 2 were bred at the first estrus following the end of treatment and gilts not returning to estrus were allowed to farrow. The remaining gilts were autopsied 10 to 40 days after the end of progestational treatment.

Trials 3 and 4 were started in order to determine whether dosages of 6-methyl-17-acetoxyprogesterone lower than 1.6 mg. would inhibit ovarian activity, 0.6 and 0.4 mg. per lb. body weight daily, respectively, being administered in the two trials. Five gilts from each trial were autopsied on the 11th and 12th day of treatment, and five gilts in each trial remained on treatment for 15 days.

A dosage of 0.5 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily for 7 to 15 days was administered in Trials 5 to 10.

[5] Gonadogen, registered trade name of The Upjohn Co., Kalamazoo, Michigan.

Results

Three gilts in Trial 1 were autopsied on the 15th day of treatment and the follicles in the ovaries did not exceed 0.5 cm. in diameter. Although the remaining 15 gilts showed conspicuous development of the external genitalia 5 to 10 days after the end of the treatment period, only 5 gilts came in estrus and were bred 5 to 9 days after the end of the treatment period. Autopsy of the five gilts 35 days following service showed that one gilt had conceived (15 normal fetuses recovered from the uteri) and that two gilts had apparently ovulated approximately 10 days previous to autopsy (10 to 12 functional C. L., estimated to be 10 days old, were present in the ovaries). Two gilts had multiple cystic ovarian conditions, the cysts varying from 1.8 to 10 cm. in diameter. Three gilts not showing estrus were autopsied 10 days after the end of treatment. One gilt apparently had ovulated without estrus and the ovaries contained 16 recently formed corpora lutea. One gilt had follicles in the ovaries 1.5 to 1.8 cm. in diameter and one gilt had ovaries containing follicles and corpora lutea less than 0.7 cm. in diameter. Three of the remaining seven gilts not showing estrus were autopsied 19 to 24 days after the end of

TABLE 2. THE INFLUENCE OF HIGH LEVELS AND CHANGING LEVELS OF 6-METHYL-17-ACETOXYPROGESTERONE, AND GONADOTROPIN ON ESTRUS AND LITER SIZE IN GILTS

Gilt no.[a]	Treatment	Gonadotropin[b]	Estrus[c] (days AET)	Normal pigs farrowed
		Trial 2—Group 1		
45	1.6 mg.[d]	1000 I.U.	3, 4	9
46	"	"	24, 25	12
47	"	"	20, 21	Open
48	"	"	None	....[f]
49	"	"	None	....[f]
50	"	"	5, 6	10
51	"	"	17, 18	Open
		Trial 2—Group 2		
52	1.6 mg. and .8 mg.[e]	None	4, 5	11
53	"	"	7, 8	4
54	"	"	1, 2	14
55	"	"	None	....[f]
56	"	1000 I.U.	4, 5	12
57	"	"	6, 7	10
58	"	"	5 to 45	....[g]

[a] Averaging 252.2 lb. in body weight.
[b] 1000 I. U. equine gonadotropin, subcutaneously, 24 hours after the end of the progestational treatment.
[c] The number of days to the start of estrus after the end of progestational treatment.
[d] 1.6 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily, by twice-a-day feeding (ADF), for 19 days.
[e] 1.6 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily, by twice-a-day feeding (ADF) from the 15th day to the end of treatment 4 days later.
[f] Did not show estrus before being removed from the experiment 35 days after the end of treatment.
[g] Constant estrus for 40 days and conceived normally at service 37 days after start of estrus.

treatment. One gilt had ovaries comparable to an immature gilt and the other two gilts had 12 to 18 regressing non-ovulatory follicles (1.8 to 2.5 cm.) in the ovaries. The remaining four gilts came into estrus 34 to 38 days after the end of treatment, and autopsy two days from the start of estrus showed that ovulation had occurred and that there was no obvious abnormalities of the genital system.

Autopsy of one gilt from Trial 2, on the 15th day of treatment, showed that follicular growth in the ovaries was minimal (less than 0.6 cm. in diameter). The gilts in Group 1, Trial 2, receiving 1000 I.U. of equine gonadotropin 24 hours after the end of progestational treatment, showed conspicuous development of the external genitalia subsequent to treatment, although only two gilts stood for service 3 and 5 days after the gonadotropin treatment (table 2). Three gilts from Group 1 stood for service after approximately one normal estrous cycle length after the end of treatment (17 to 24 days).

Six of seven gilts in Group 2, of Trial 2, in which the progestational dosage was reduced during treatment to 0.8 mg. per lb. body weight daily and in which three gilts received gonadotropin, came into estrus 1 to 7 days after the end of treatment (table 2). Five of the six gilts conceived at the controlled estrus and the 6th gilt remained in estrus for 40 days.

The average number of pigs farrowed by gilts bred subsequent to treatment with 6-methyl-17-acetoxyprogesterone, in Trial 2, (10.3—average pig weight 2.47 lb.) compares favorably with the number of pigs farrowed by the 13 control gilts carried to term (10.5—average pig weight 2.46 lb.). The percentage of control pigs (95%) conceiving within the same period of time (30 days) was greater than in the treated group (57%).

Follicular development was inhibited in the ovaries of gilts receiving 0.6 and 0.4 mg. of 6-methyl-17-acetoxyprogesterone, for 11 and 12 days, respectively (table 3). The follicles

TABLE 3. FOLLICULAR DEVELOPMENT AND CORPORA LUTEA IN THE OVARIES OF GILTS DURING ORAL ADMINISTRATION OF 6-METHYL-17-ACETOXYPROGESTERONE

Gilt no.	Weight (lb.)	Treatment[a] (mg./lb./day)	Autopsy (days on treatment)	Ovarian condition[b]	
				Follicles	Corpora lutea
			Trial 3		
60	246	0.6	11	Numerous 0.4 cm.	15; 0.7 cm.
61	252	.6	11	" 0.6 cm.	8; 0.9 cm.
62	230	.6	11	5 1.2 cm.	13; 1.2 cm.
63	273	.6	11	2 1.3 cm.	9; 1.3 cm.
64	236	.6	11	Numerous 0.7 cm.	13; 0.6 cm.
			Trial 4		
70	244	.4	12	Numerous 0.5 cm.	10; 0.9 cm.
71	229	.4	12	" 0.7 cm.	less 0.5 cm.
72	211	.4	12	" 0.5 cm.	16; 0.1 cm.
73	201	.4	12	" 0.6 cm.	less 0.5 cm.
74	209	.4	12	" 0.6 cm.	11; 0.9 cm.

[a] Gilts from Trials 3 and 4 fed 0.6 or 0.4 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily, by twice-a-day feeding (ADF), and autopsied on the 11 and 12 days of treatment, respectively.
[b] The number and the largest sizes of follicles and corpora lutea in the ovaries at autopsy.

TABLE 4. ESTRUS AND FERTILITY SUBSEQUENT TO THE ORAL ADMINISTRATION OF 6-METHYL-17-ACETOXYPROGESTERONE IN GILTS [a]

Gilt no.	Weight (lb.)	Estrus [b] (days AET)	Estrus [c] (days AET)	Autopsy results [d]	
				Functional C.L.	Fetuses recovered
			Trial 3		
65	233	4, 5	.....	13	13
66	274	4, 5, 6	.....	11	11
67	254	4, 5, 6	.....	16	9
68	243	4, 5	23, 24	16	12 [e]
69	220	4, 5. 6	.....	12	9
			Trial 4		
75	250	4, 5, 6	.....	15	12
76	242	5, 6	23, 24	11	11 [e]
77	245	5, 6	23, 24	14	5 [e]
78	260	5. 6	23, 24	11 [f]	Open
79	206	5. 6	.....	11	8

[a] Gilts from Trials 3 and 4 fed 0.6 or 0.4 cm. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily, by twice-a-day feeding (ADF), for 15 days, respectively.
[b] The number of days to the first estrus after the end of treatment. The gilts were bred where the day of estrus is underlined.
[c] The number of days to the second estrus after the end of treatment in gilts not bred or returning to estrus after breeding.
[d] Functional corpora lutea in the ovaries and fetuses recovered from the uteri 35 days from service. The gilts in Trials 6 and 7 served each day of estrus and the gilts in Trials 8 and 9 served once the second day of estrus.
[e] The corpora lutea in the ovaries and fetuses recovered are the product of the second estrus following the end of treatment.
[f] The corpora lutea from the third estrus following the end of treatment.

in the ovaries of two gilts (1.2 and 1.3 cm. in diameter) were considered regressing and the presence of small follicles (0.4–0.7 cm. in diameter) in the ovaries of gilts in which the corpora had declined (0.6 to 0.9 cm. in diameter) was indicative of complete suppression of post-luteal follicular growth.

Five gilts each from Trials 3 and 4 remained on progestational treatment for 15 days and came into estrus 4 to 5 days after the end of treatment. The gilts were bred each day of estrus and four gilts returned to estrus 23 days from the end of treatment (table 4). One gilt (No. 78) failed to conceive at the second estrus following the end of treatment and autopsy 10 days after the third estrus following the end of treatment did not reveal any apparent abnormalities of the genital system.

The gilts in Trials 5, 6, 7 and 8 were fed 0.5 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily for 15 days. Thirty-two of 36 gilts (88.8%) came into estrus 4 to 5 days after the end of treatment (table 5) and 17 of 22 gilts (77.3%) bred at the controlled estrus, 4 to 5 days after the end of treatment, conceived (table 5). Three gilts, although showing extreme development of the external genitalia, would not stand for service 4 to 5 days after the end of treatment and came into estrus 19 to 22 days after the end of treatment. One gilt (No. 106) did not show estrous activity before autopsy 35 days after the end of treatment, and the ovaries contained 12 cystic follicles. Gilts 113 and 114 were bred at estrus 5 and 6 days after the end of treatment and had not returned to estrus before autopsy 35 days after the end of treatment. Gilt 113 was not pregnant at autopsy and the genital tract appeared normal (the ovaries containing 12 functional corpora lutea). Gilt 114 was not pregnant at autopsy and the ovaries contained 13 cystic follicles. Eleven of the gilts returning to estrus and three gilts showing estrus for the first time 19 to 22 days after the end of treatment were bred and autopsied 35 days from service. Twelve gilts were pregnant at autopsy (85.7%).

The average amount of ration fed in Trials 3 to 8 averaged 3 to 4 lb. per gilt daily. The gilts in Trial 9 were fed 0.5 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily (ADF) in an average daily ration of 7 lb. per gilt. One gilt came into estrus on the 5th day of treament (table 6), the first instance in the study of a gilt showing estrus while on treatment. The remaining gilts came into estrus 3 to 14 days after the end of treatment. Gilt 124, that showed estrus during treatment, was autopsied 17 days after the end of treatment. The ovaries contained 17 large fluid-filled luteinized sacs (1.8 to 3 cm. in

diameter) and 10 normal appearing follicles of preovulatory size. Six of the eight gilts conceived at the first estrus after the end of treatment and autopsy of the two gilts returning to estrus did not reveal any apparent genital tract abnormalities.

The gilts in Trial 10 received the same progestational dosage and total amount of ration

TABLE 5. CONTROLLED ESTRUS AND FERTILITY IN GILTS SUBSEQUENT TO ORAL ADMINISTRATION OF 6-METHYL-17-ACETOXYPROGESTERONE

Gilt no.	Weight (lb.)	Estrus[a] (days AET)	Estrus[b] (days AET)	Autopsy results[c]	
				Functional C.L.	Fetuses recovered
			Trial 5		
80	260	4, 5	.....	18	14
81	292	4, 5	.....	21	12
82	207	4, 5	.....	11	7
83	293	4, 5	.....	19	15
84	250	4, 5	23, 24	18	14
85	225	4, 5	23, 24	15	14
86	267	4, 5, 6	23, 24, 25	15	13
87	218	5, 6	23, 24	13	11
88	218	5, 6	23, 24	12	13
			Trial 6		
89	291	4, 5, 6	.....	13	12
90	250	5, 6	.....	11	9
91	273	4, 5	.....	13	11
92	250	4, 5	.....	12	11
93	234	4, 5	.....	11	11
94	268	4, 5	.....	15	12
95	273	...[d]	22, 23	15	13
96	295	4, 5	22, 23	14[e]	Open
97	246	...[d]	22, 23	15[e]	Open
			Trial 7		
98	253	4, 5	.....	7	8
99	253	5, 6	.....	12	7
100	246	5, 6	.....	13	11
101	270	4, 5	23, 24	14	11
102	283	5, 6	23, 24	14	9
103	261	4, 5	21, 22, 23	14[e]	Open
104	236	4, 6	25, 26	14	14
105	255	5, 6	24, 25	13	13
106	269	...	.....	cystic ovarian condition	
			Trial 8		
107	279	5, 6	.....	14	12
108	283	4, 5	.....	24	10
109	302	4, 5	.....	16	15
110	310	4, 5	.....	16	12
111	285	5, 6	23, 24	pyometria—not bred	
112	274	4, 5	23, 24	12	11
113	294	5, 6	.....	12[e]	Open
114	281	5, 6	.....	cystic ovarian condition	
115	295	...[d]	19, 20	13	10

[a] The number of days to the first estrus after the end of treatment. The gilts were serviced when the day of estrus is underlined.
[b] The number of days to the second estrus after the end of treatment. The gilts were serviced when the day of estrus is underlined.
[c] Thirty-five days after the last service.
[d] Would not stand for service 4 to 5 days after the end of treatment.
[e] The gilts were not pregnant and the corpora lutea listed are the product of the third estrus after the end of treatment.

as the gilts in Trial 9 but the duration of treatment was extended to 15 days. The majority of the gilts in Trial 10 showed conspicuous development of the external genitalia 2 to 7 days after the end of treatment and six gilts showed estrus 3 to 4 days after the end of treatment (table 6). Only one of the five gilts bred at the controlled estrus conceived. Four of the gilts showed estrus for the first time and four of the gilts recycled 19 to 26 days from the end of treatment.

Gilts conceiving subsequent to progestational treatment in Trials 3 to 10 were autopsied 35 days from service. Twenty-six mature gilts were bred at estrus and autopsied 35 to 45 days from service in order to compare the ovulation rate and fetal recovery to that obtained in gilts subsequent to treatment. Twenty of the control gilts conceived at the first service. The ovulation rate at estrus, as estimated by the number of functional corpora lutea at 35 to 45 days of pregnancy, averaged 12.7 ± 2.50 per gilt. The number of fetuses recovered from the uteri averaged 9.2 ± 1.80 per gilt.

Six gilts exhibited congenital abnormalities at autopsy and were excluded from the data. One was a control gilt and five were from treated groups. The abnormalities, in each case, were incomplete fusion of the Müllerian ducts with resultant blind pouches with varying amounts of trapped fluids. The uteri were reduced to thin walled vesicles in the areas of fluid accumulation.

Discussion

Apparently 1.6 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily, when the compound was mixed with the normal ration, provided a progestational inhibition of ovarian activity from which the gilts did not readily rebound after termination of treat-

TABLE 6. THE INFLUENCE OF INCREASED DAILY RATION ON THE ORAL EFFECTIVENESS OF 6-METHYL-17-ACETOXYPROGESTERONE IN GILTS

Gilt no.	Weight (lb.)	Estrus[a] (days AET)	Estrus[b] (days AET)	Autopsy results[c] Functional C.L.	Fetuses recovered
			Trial 9[d]		
116	...	7, 8	27, 28	14[e]	Open
117	...	4, 5	.....	15	8
118	...	3, 4	.....	11	2
119	...	3, 4	.....	14	14
120	...	4, 5	.....	13	9
121	...	6, 7	.....	11	11
122	...	14, 15	.....	12	12
123	...	9, 10	29, 30	13[e]	Open
124	...	...	.....	17	luteinized follicles[f]
			Trial 10		
125	254	4, 5	.....	26	14
126	273	3, 4	.....	10	Open[g]
127	279	4, 5	23, 24	16	16
128	277	3, 4	21, 22	17	16
129	281	3, 4	25, 26	17	10
130	263	4, 5	22, 23, 24	12	11
131	265	...[h]	23, 24	9	9
132	298	...[h]	19, 20	12	8
133	285	...[h]	26, 27	21	19
134	286	...[h]	25, 26	13	9

[a] The number of days to the first estrus after the end of treatment. The gilts were serviced when the day of estrus is underlined.
[b] The number of days to the second estrus after the end of treatment. The gilts were serviced when the day of estrus is underlined.
[c] Thirty-five days after the last service.
[d] Gilts 116 through 124, inclusive, averaged 253 lb. in body weight.
[e] Corpora lutea listed resulted from ovulation at the second estrus after the end of treatment.
[f] Gilt showed estrus on the fifth day of treatment. Numerous preovulatory follicles in ovaries at autopsy 17 days after the end of treatment.
[g] Second post-treatment estrus not apparent and was in late luteal phase of estrous cycle at autopsy.
[h] The gilts did not exhibit estrus 3 to 5 days after the end of treatment, although they exhibited extreme development of external genitalia at that time.

ment (Trial 1). The inhibition of follicular growth was complete by the 15th day of treatment, as judged by the absence of follicles greater than 0.5 cm. in diameter in the ovaries. The length of time after the end of treatment to estrus (5 to 9 days) suggested that the follicular growth phase subsequent to progestational treatment was delayed. Autopsy of gilts following treatment demonstrated that the delayed follicular growth phase terminated in estrus and ovulation, or ovulation without estrus and recycling 34 to 38 days after the end of treatment or the development of follicles that either became cystic or luteinized.

The reduction in the amount of 6-methyl-17-acetoxyprogesterone from 1.6 to 0.8 mg. per lb. body weight daily (ADF) on the 15th day of treatment resulted, in at least one instance (Gilt 54, Trial 2), in escape from progestational inhibition. The injection of 1000 I.U. of equine gonadotropin, one day after the end of excessive progestational treatment, apparently facilitated ovulation. This was indicated by the shorter interval from the end of treatment to estrus (3 to 6 days as compared to 5 to 9 days in Trial 1) and recycling of 3 gilts 17 to 24 days after the end of treatment (as compared to 34 to 38 days in Trial 1), presumably the result of ovulation without estrus 3 to 4 days after the end of treatment. The injection of 1000 I.U. of equine gonadotropin subsequent to progestational inhibition did not result in superovulation.

The results of Trials 3 and 4 demonstrated that dosage levels of 0.4 to 0.6 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight (ADF) inhibited follicular growth, and yet not to the extent that the gilts could not readily rebound following treatment. The occurrence of estrus and ovulation, 4 to 5 days after the end of progestational treatment, was comparable to the post-treatment interval to estrus in earlier studies (Nellor, 1960) in which progestational inhibition was considered just adequate to inhibit follicular growth.

The dosage level of 0.5 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily (ADF) was selected in Trials 5 through 8 in order to allow for variations in food intake between gilts and yet to stay within the dosage range utilized in Trials 3 and 4 (0.4 to 0.6 mg.). The gilts were consuming an average of 6 lb. of ration daily prior to treatment. The reduction in ration to an average of 3 to 4 lb. daily resulted in considerable activity at feeding time, and the ration containing 6-methyl-17-acetoxyprogesterone was consumed within 15 minutes. Under competitive feeding conditions an average weight gilt would have had to consume less than 2.5 or more than 3.5 lb. of the ration daily in order to receive less or exceed 0.4 to 0.6 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily. This was not likely during the limited competitive feeding time.

The results of Trials 5, 6, 7 and 8 demonstrated that a high degree of control of the time of estrus can be obtained with this regime. Eighty-nine per cent of the treated gilts came into estrus 4 to 5 days after the end of progestational treatment. Gilts not showing estrus 4 to 5 days after the end of treatment came into estrus 19 to 22 days after the end of treatment, that is, one normal estrous cycle length after the end of treatment. The majority of gilts not conceiving at the controlled estrus returned to estrus 22 to 25 days after the end of treatment. The imposed cycle is retained in gilts either not bred at the controlled estrus or in gilts returning to estrus following breeding at the controlled estrus, that is, the range in the start of the second estrus following the end of treatment is 4 days, as compared to 2 days at the first estrus following the end of treatment.

Effective control of the time of estrus was not obtained when gilts were fed 0.5 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily (ADF), in an average of 7 lb. of ration daily. The results in Trial 9 are what could be expected if an inadequate progestational dosage was administered. One gilt (No. 124) came into estrus on the 5th day of treatment and apparently was in the early follicular phase of the estrous cycle when placed on treatment. Autopsy 17 days after the end of treatment demonstrated that ovulation had not occurred at the estrus during treatment and that the follicles developed past pre-ovulatory size and luteinized. The large luteinized sacs apparently regulated the time of the next follicular growth phase since the gilt was in the follicular phase at autopsy, 17 days after the end of treatment, or 19 days after the time of estrus during treatment. Four of the remaining gilts came into estrus 3 to 4 days after the end of treatment and are assumed to have been on the 9th to 12th day of the estrous cycle when placed on treatment. It is suggested that the occurrence of estrus 6, 7, 9 and 14 days after the end of treatment represents regulation of estrus by non-inhibited luteal tissue in the ovaries of gilts placed on trial during the early luteal phase of the estrous cycle.

The gilt showing estrus during treatment and the occurrence of estrus 3 days after the end of treatment in two gilts in Trial 9 describes an inadequate progestational dosage. In Trial 10, in which the dosage level was the same as in Trial 9 but the duration of treatment was 15 days, three of six gilts came into estrus 3 days (60 hours) after the end of treatment. Only one of five gilts bred at the controlled estrus conceived, and this gilt apparently superovulated at the controlled estrus (26 functional corpora lutea were in the ovaries at 35 days of pregnancy). It is suggested that the occurrence of estrus less than 60 hours after the end of progestational treatment is associated with inadequate progestational inhibition and an accelerated follicular growth phase, resulting in a higher incidence of ovulation without estrus and a low incidence of fertility in gilts showing estrus.

The interval from the last injection of progesterone to the start of estrus in heifers was reported to decrease from 5.2 days as dosage of injected progesterone decreased (Ulberg *et al.*, 1951). Anderson and Melampy (1959) reported that increasing the amount of orally administered norethynodrel and ethynylestradiol 3-methyl ether (from 4 to 7.5 or 15 mg. per 100 lb. body weight daily) resulted in a slightly longer delay in the time of the normal estrus in gilts. The inadequate progestational inhibition in the present study was associated with increased amounts of ration offered. It was noted that when the ration was increased to an average of 7 lb. daily per gilt, that some gilts left the trough before the entire ration was consumed. For this reason the unfavorable results obtained with an increased amount of ration, when the amount of 6-methyl-17-acetoxyprogesterone per lb. body weight (ADF) remains the same, could either be due to considerable variations in consumption from the average amount of ration offered, or to a decreased availability of 6-methyl-17-acetoxyprogesterone within the ration.

The autopsy results of these studies (table 3) and the results obtained with short duration of treatment (table 6) indicate that amounts of 6-methyl-17-acetoxyprogesterone capable of inhibiting follicular development do not inhibit the development or the functional life of corpora lutea present in the ovaries of normal cycling gilts. Sammelwitz and Nalbandov (1958) reported that the injection of progesterone does not appear to alter significantly normal luteal development or regression in cycling gilts. The duration of treatment with 6-methyl-17-acetoxyprogesterone in swine should therefore exceed the developmental and functional life of the corpora lutea. The treatment duration of 15 days seems adequate for this purpose.

The conception rate at estrus subsequent to progestational treatment was dependent upon the degree of ovarian inhibition induced during treatment. The fertility, as estimated by the numbers of pigs born alive or the number of normal fetuses recovered at 35 days pregnancy, was not depressed by progestational treatment, and compares favorably with the control gilts. Dosage levels of 1.6 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily (ADF), in an average daily ration of 5 lb. per gilt (Trials 1 and 2), and 0.5 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily (ADF), in an average of 7 lb. of ration per gilt (Trials 9 and 10), were considered excessive and inadequate, respectively, and were associated with a low conception rate. When 0.4 to 0.6 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily (ADF) were fed in an average of 3 to 4 lb. of ration daily (Trials 3 through 8 considered to be the optimal dosage with a limited ration), the ovulation rate at the controlled estrus (14.09), as estimated by the functional corpora lutea at 35 days of pregnancy, was not significantly different from the control gilts (12.7), and the number of normal fetuses recovered at 35 days of pregnancy was not significantly different in the treated gilts (10.91 per gilt) from the control gilts (9.21 per gilt).

The ovulation rate in gilts not showing estrus until 19 to 26 days after the end of treatment and in gilts returning to estrus 21 to 25 days after the end of treatment (Trials 3 to 10), as estimated by the number of corpora lutea in the ovaries at 35 days of pregnancy, was 14.2 per gilt; and the number of fetuses recovered from the tracts was 11.8 per gilt. The conception rate at estrus 19 to 26 days after the end of treatment (88%) was greater than the conception rate in gilts bred 3 to 7 days after the end of treatment (69.8%—Trials 3 to 10).

There are reports in the literature (Baker *et al.*, 1954; Trimberger and Hansel, 1955) on increased incidence estrous cycle abnormalities in domestic animals following progesterone treatment. The results of the present study and of an earlier study (Nellor, 1960) demonstrate that cystic ovaries can result from inadequate or excessive progestational

treatment. The cysts can vary from thin walled follicular cysts, varying in diameter from 1.8 to 10 cm., with no evidence of luteinization, to large lutenized fluid filled sacs with a few layers of luteal tissue to almost complete filling with luteal tissue (2.0 to 4 cm. in diameter). It would seem that the eventual fate of large cystic follicles, in gilts that eventually return to estrus, is replacement of the walls with luteal tissue. Gilts on inadequate progestational treatment may develop slow growing follicles which while under treatment do not approach extremely large size (2 to 4 cm.), but which readily luteinize upon cessation of treatment. They cannot be classified as luteal cysts since their functional life rarely exceeds the life of normal corpora lutea. The development of cysts subsequent to excessive progestational treatment is associated with a delayed follicular growth phase, and usually terminates in extremely large fluid filled sacs, which may or may not be associated with prolonged or excessive estrous behavior. The injection of gonadotropin can facilitate the follicular growth phase and reduce the incidence of cystic follicle formation subsequent to excessive progestational treatment.

There was a high incidence of cysts in the present study (Trial 1) where the progestational dosage was considered excessive. The incidence of follicular cysts was very low where the progestational dosage was considered optimal (Trials 3 through 8). Two cases of follicular cysts were observed in 36 gilts included in these trials. An incidence of 5.3% of cystic ovaries in mature unselected gilts, where nothing is known of previous estrous cycle activity, is not considered abnormal. Two of the 26 control gilts (7.7%) autopsied 45 days following service exhibited follicular cysts in the ovaries.

Summary

Trials were conducted with 149 gilts, 41 control gilts and 108 gilts fed from 0.4 to 1.6 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily, by twice-a-day feeding, in an average of 3 to 7 lb. of ration daily per gilt. The duration of treatment was from 7 to 27 days. The gilts ranged from 190 to 310 lb. in body weight.

The amount of ration offered influenced the ability to control accurately the time of estrus and ovulation in a group of treated gilts fed together. A high degree of control of the time of estrus and ovulation was obtained when gilts were fed 0.5 mg. of 6-methyl-17-acetoxyprogesterone per lb. body weight daily, by twice-a-day feeding for 15 days, in a limited amount of daily ration (an average of 3 to 4 lb. daily per gilt). Eighty-nine percent of the gilts came into estrus 4 to 5 days after the end of progestational treatment. The conception rate in gilts bred at the controlled estrus was 77%. The estimated ovulation rate at the controlled estrus (14.5 per gilt) and the number of fetuses recovered 35 days following service at the controlled estrus (11.1 per gilt) were not significantly greater than in comparable age and weight control gilts (11.5 and 9.3, respectively).

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SWINE HEMATOLOGY FROM BIRTH TO MATURITY. I. SERUM PROTEINS [1]

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IN biological research with any species of animals one is concerned with normal values of various biochemical and biophysical measures for purposes of comparison with the particular segment of the population with which he may be working. Such is the case with hematological measures in swine, and, because one is concerned with these measures at all ages of the species, it is important to know the changes which normally occur from birth to maturity.

A relatively large number of studies of the protein composition of swine serum and plasma have been published, many of these dealing with the development of separatory procedures (Svensson, 1941; Cooper, 1945; Moore, 1945a; Koenig and Hogness, 1946) or with certain storage effects (Mathews and Buthala, 1956; Pensinger *et al.*, 1959). Other studies have been concerned with swine serum or plasma proteins of normal or experimental animals within a limited age range, utilizing a small number of animals in nutrient deficiencies (Miller *et al.*, 1957; Hoefer *et al.*, 1960; Smith *et al.*, 1960) or in disease conditions (Vesselinovitch, 1955). Electrophoretic analyses have been reported of the serum or plasma of swine fetuses (Moore *et al.*, 1945b; Rutqvist, 1958), young pigs (Foster *et al.*, 1951; Nordbring and Olsson, 1957, 1958a, b; Olsson, 1959b; Vesselinovitch, 1959; Lecce and Matrone, 1960), slaughter age animals (Svensson, 1941; Moore, 1945a; Deutsch and Goodloe, 1945; Koenig and Hogness, 1946; Cartwright *et al.*, 1948; Boguth, 1953; Hill and Schumann, 1953; Mathews and Buthala, 1955; Nordbring and Olsson, 1957; Knill *et al.*, 1958; Haaranen, 1960) and adult female swine during various stages of gestation and lactation (Foster *et al.*, 1950; Friedell *et al.*, 1951; Nordbring and Olsson, 1957). Jacobsen and Moustgaard (1950) have studied the ammonium sulfate fractionation of serum proteins of Danish swine from birth to six months of age. Rook *et al.* (1951) reported the electrophoretic changes in serum proteins of similar animals over this age range with fractionation by moving boundary electrophoresis on serum from a very limited number of animals at each age. Only the more recent studies have used paper electrophoresis to accomplish serum or plasma protein separation. Knill *et al.* (1958) have shown that this procedure yields statistically valid protein concentration values. The rapidity of determinations using small quantities of serum or plasma provides a definite advantage for this technique over moving boundary electrophoresis for routine purposes.

The present study was conducted to establish normal values for serum proteins of swine from birth to maturity and to observe changes in the serum protein electrophoretic patterns as determined by the paper strip zone electrophoresis technique.

Methods

Over 1,100 serum samples were obtained from healthy swine of all ages from the Michigan State University swine herd over a period of 2 years (1957–1959). Most of the animals were purebred Duroc or Hampshires or a cross of the two breeds. All animals were reared in confinement. Pigs were weaned at 5 or 6 weeks of age and reared to maturity on natural diets. Blood samples were obtained from the anterior vena cava of all animals by the technique described by Carle and Dewhirst (1942) and placed in centrifuge tubes. After clotting, the blood was "rimmed" in the tube and clot contraction was accelerated by placing the tubes in a temperature environment of 40° C. for 30 minutes. Separation of serum and clot was completed in a Servall, model M, medium angle centrifuge at 3000 rpm for 15 minutes. Serum was then withdrawn from the tubes, placed in vials and frozen. The samples were stored in a frozen state at −23° C. and were thawed to room temperature (23° C.) immediately preceding electrophoretic separation. Pensinger *et al.*

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(1959) have stated that freezing and thawing significantly alters the protein pattern of baby pig serum and that fresh serum should be analyzed in preference to frozen. Mathews and Buthala (1956), on the other hand, found that repeated freezing and thawing of swine serum did not alter the electrophoretic pattern. In our own studies (unpublished data) of serum storage and handling procedures, a single freezing and thawing has not significantly changed the electrophoretic pattern.

Electrophoretic separation was accomplished on a Spinco, model R, paper electrophoresis system (Spinco Technical Bulletin 6027A) at room temperature. A constant current of 2.5 milliamperes per cell was maintained for 16 hours on Schleicher and Schuell 2043A paper strips, veronal buffer being used of pH 8.6 and an ionic strength of 0.075. Relative intensities of the separated proteins were determined from the uptake of bromphenol blue dye by scanning the stained strips with the Spinco model RB Analytrol. A certain amount of subjective judgment was required to exact a separation of the β- and γ-globulin components from the patterns of the scanned strips in serum of pigs 6 to 48 hours of age. Rutqvist (1958) and Nordbring and Olsson (1957) also encountered this difficulty. Total serum protein was determined by the biuret procedure of Reiner (1953). Pooled porcine serum was standardized by macro-Kjeldahl methods. Duplicate determinations of total serum protein and electrophoretic pattern were made on each serum sample.

Results and Discussion

The means together with their standard errors for total serum protein and the relative percentages of the electrophoretically separated components are presented in table 1. These values and the comparative changes in serum protein fractions which occur with age of swine may be more readily perceived from figure 1.

The concentration of serum protein in the newborn pig is about 2 gm. per 100 ml.

TABLE 1. TOTAL SERUM PROTEIN AND SERUM PROTEIN DISTRIBUTION IN SWINE FROM BIRTH TO MATURITY

Age	No. of animals	Total serum protein (gm./100 ml.)	Distribution of serum proteins (%)			
			γ-globulin	β-globulin	α-globulin	Albumin
0 hours	47	2.22(.04)*	6.5(0.5)	15.9(0.9)	60.8(1.9)	16.7(1.3)
6 hours	26	3.67(.23)	31.7(1.9)	23.7(1.1)	34.8(2.1)	9.5(1.4)
12 hours	18	5.67(.34)	43.3(1.8)	24.4(1.5)	24.4(2.4)	8.0(0.7)
18 hours	25	5.24(.30)	44.7(1.8)	21.9(1.1)	26.4(2.2)	7.0(0.4)
24 hours	36	5.20(.21)	46.3(1.4)	21.3(0.9)	23.4(1.6)	8.9(0.5)
30 hours	15	5.21(.45)	41.5(2.3)	24.6(1.6)	23.8(2.7)	10.2(0.9)
36 hours	11	5.84(.38)	42.6(1.7)	23.3(1.7)	22.5(1.8)	11.5(1.0)
42 hours	9	5.94(.51)	41.4(2.0)	25.4(1.0)	20.9(1.9)	12.3(1.4)
48 hours	30	5.30(.22)	40.9(1.1)	21.5(0.8)	22.8(1.3)	14.8(0.5)
3 and 4 days	45	5.07(.15)	35.9(0.8)	15.9(0.6)	32.3(1.3)	15.0(0.6)
7 days	47	4.78(.15)	25.2(1.1)	17.5(0.5)	25.6(1.0)	31.6(0.9)
10 and 11 days	37	4.76(.11)	15.3(0.9)	20.3(0.7)	29.0(1.2)	35.4(1.2)
2 weeks	34	4.78(.10)	14.6(0.9)	17.5(0.5)	23.5(1.1)	44.3(1.3)
3 weeks	94	4.75(.06)	9.8(0.1)	18.5(0.4)	24.1(0.2)	47.6(0.7)
4 weeks	71	4.83(.06)	9.6(0.6)	19.0(0.4)	25.7(0.5)	45.7(0.7)
5 weeks	71	4.76(.06)	7.6(0.3)	18.9(0.4)	26.1(0.5)	47.4(0.7)
6 weeks	60	5.30(.12)	9.1(0.4)	18.6(0.4)	25.3(0.6)	47.0(0.8)
7 weeks	33	4.93(.12)	9.1(0.4)	18.2(0.4)	24.4(0.8)	48.2(1.1)
9 weeks	84	5.41(.06)	14.4(0.5)	15.9(0.4)	28.8(0.4)	40.9(0.5)
3 months	36	5.68(.09)	20.0(0.8)	16.0(0.5)	28.7(0.7)	45.3(0.8)
4 months	73	6.78(.06)	23.1(0.6)	13.1(0.4)	23.1(0.4)	41.0(0.7)
5 months	67	6.89(.38)	22.7(0.6)	14.0(0.4)	21.2(0.5)	42.2(1.0)
6 months	76	6.80(.05)	19.3(0.6)	12.6(0.2)	19.4(0.3)	48.6(0.7)
1 year[a]	11	7.51(.10)	16.4(0.9)	12.6(0.7)	18.2(0.7)	52.7(0.6)
1 year[b]	21	7.51(.09)	16.6(0.8)	13.3(0.7)	18.0(0.6)	52.1(1.4)
2 years[a]	13	7.97(.17)	19.1(1.0)	13.1(0.7)	16.7(0.5)	51.0(1.0)
2 years[b]	12	7.61(.09)	16.8(1.3)	13.3(0.5)	17.1(0.9)	52.7(1.2)

* Standard error of mean.
[a] Gilts or sows in mid-gestation.
[b] Late gestation.

of serum composed mainly of α_2-globulin with much smaller percentages of the other components. Rook *et al.* (1951) and Rutqvist (1958) found no γ-globulin present in the serum of pigs at birth; however, Foster *et al.* (1950) reported a small percentage and Nordbring and Olsson (1957) obtained an average of 7.9% γ-globulin in the serum proteins of newborn pigs. A similar percentage of slow moving components was consistently found at birth in pigs in the present study. The absolute amount of this component present at birth, however, is extremely low because of the small amount of total serum proteins. As the pig suckles and consumes colostrum his

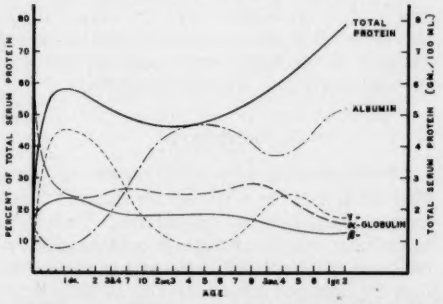

Figure 1. Changes in total serum protein and serum protein fractions occurring with age from birth to maturity.

γ-globulin increases rapidly and together with the increase in β-globulin accounts for most of the increase in the total serum protein. Foster *et al.* (1950) have shown that the colostral whey of the sow at parturition contains a high percentage of slow moving components which diminish rapidly through progressive stages of lactation. Laskowski *et al.* (1957) have demonstrated the presence of a high level of trypsin inhibitor in early colostrum which is rapidly reduced in the first few days of lactation. Nordbring and Olsson (1958a, b) have shown that bovine trypsin inhibitor has a positive although highly variable influence upon the degree of absorption of immune globulins from porcine colostrum.

The level of β- and γ-globulins and the total serum protein reach a maximum within the first 24 hours after birth. Nordbring and Olsson (1957) and Olsson (1959b) have shown that pigs absorb large amounts of antibodies or immune globulins during the first 12 hours after birth. Speer *et al.* (1957) have demonstrated that the absorption of ingested antibodies by the pig occurs only within the first 24 hours after birth, the absorption rate diminishing exponentially from birth. It is evident in the present study that the level of total serum protein reaches an early maximum at 12 hours after birth. This value then diminishes somewhat but remains relatively constant until three months and from this age until maturity a gradual increase occurs.

The serum γ-globulin diminishes rapidly from a maximum of 46% present at 24 hours after birth to a near minimum at 3 weeks of age. The values of γ-globulin level at any age in this age interval form a straight line on a semi-logarithmic plot fitting the following equation:

$$\gamma_1 = \gamma_0 e^{-.077t}$$

in which γ_0 = the level of serum γ-globulin at 24 hours of age, γ_1 = the level of serum γ-globulin at any age between 24 hours and 3 weeks, and t = time in days from the 24 hour maximum. The half life of the serum γ-globulin present in the baby pig as a result of colostral absorption is

$$t\tfrac{1}{2} = \frac{\ln 2}{k} = \frac{.693}{.077} = 9 \text{ days.}$$

This is somewhat slower than the turnover rate of rat serum γ-globulin obtained by Jeffay and Winzler (1958) but indicates an exponential rate of γ-globulin extinction from the serum of the pig with little or no production of γ-globulins by the baby pig through the first three weeks of life. This observation is supported by the observed failure of baby pigs to make a measurable serum antibody response to an intraperitoneally injected antigen (Staub and Boguth, 1956; Hoerlein, 1957; Harmon *et al.*, 1959; Olsson, 1959a) and by the absence of γ-globulin in the serum colostrum, for the first few weeks of life of pigs, raised artificially without receiving (Bauriedel *et al.*, 1954; Staub and Boguth, 1956; Hoerlein, 1957). Maximum production of serum γ-globulin in the present study occurred between the ages of 2 and 6 months.

The production of serum albumin, on the other hand, begins very rapidly after birth, maximum increase in its production occurring during the period of rapid extinction of colostral obtained serum γ-globulin. The level of serum albumin remains rather constant throughout life after reaching a maximum at about 3 weeks of age and is largely responsible for the maintenance of plasma volume.

The amount of serum α-globulins is quite constant throughout life. There is a great percentage reduction in this component in the first 24 hours after birth as a result of the absorption of immune globulins, but the total amount varies only slightly. The presence of two distinct fractions of this component (α_1 and α_2) is observable in the electrophoretic patterns (figure 2) of the serum proteins from

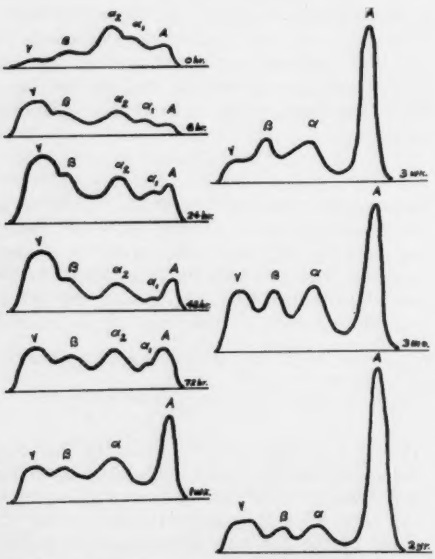

Figure 2. Electrophoretic patterns of the serum proteins of swine of different ages.

pigs the first few days of life. The α_1-globulin fraction becomes indistinguishable from serum albumin as the pig advances in age. This phenomenon has been observed by Nordbring and Olsson (1957). Rook *et al.* (1951) referred to the α_1-globulin fraction as component "X". Both of these authors suggest that this fraction may be largely composed of fetuin, a mucoprotein which Pedersen (1944) first found in high concentration in fetal calf sera. Deutsch (1954) observed that in a veronal buffer of pH 8.6 this protein migrated to the α-globulin area and constituted 45% of the serum proteins in the fetal calf. An important protein in the α_2-globulin fraction is ceruloplasmin, a copper containing oxidase isolated by Holmberg and Laurell (1948).

Following the initial increase in serum β-globulin due to absorption from colostrum this component remains at a fairly constant level to maturity. Surgenor *et al.* (1949) isolated transferrin, the plasma iron binding and transport protein, from the β_1-globulin fraction of human plasma. It is possible that this specific protein might constitute a substantial portion of the serum β-globulins obtained from colostrum and provide an increased serum iron binding capacity during the period immediately following birth.

The changes in electrophoretic pattern with advancing age are shown in figure 2. The increase in β- and γ-globulin through the first 24 hours after birth and the incomplete separation of these two components in serum of pigs 6 to 48 hours of age are characteristic. The α_1-globulin fraction present at birth is no longer observable after the pigs are 72 hours old. From this age to maturity the principal changes in electrophoretic pattern occur in the γ-globulin and albumin fractions.

Summary

Electrophoretic and total protein analyses were made on over 1,100 serum samples taken from swine at frequent age intervals from birth to maturity. Rapid changes in total serum protein and electrophoretic pattern occur in the first 24 hours of life in the pig as a result of colostral absorption of β- and γ-globulins. Rapid changes also occur in the subsequent three-week period because of the catabolism of serum γ-globulin, the immediate anabolism of serum albumin and the absence of a significant amount of serum γ-globulin production. Maximum production of serum γ-globulin occurs between the ages of 2 and 6 months. Values of serum α- and β-globulins after the neonatal changes are relatively constant throughout life.

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NUTRIENT INTAKE AND LIVESTOCK RESPONSES ON SEEDED FOOTHILL RANGES

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DEFICIENCY of suitable spring range in the Intermountain area generally is a limiting factor for successful livestock production. During recent years, livestock men have shown increased interest in artificial rehabilitation of depleted foothill range to provide more forage.

Considerable uncertainty exists as to the relative grazing value of the species available for planting on arid ranges. Because of differences in palatability, nutritive value, and growth habits, all species are not equally valuable for various seasons of grazing and kinds of livestock. This study was conducted to compare nutritive values of four major wheatgrass species and to measure livestock responses (figures 1 and 2). The species studied

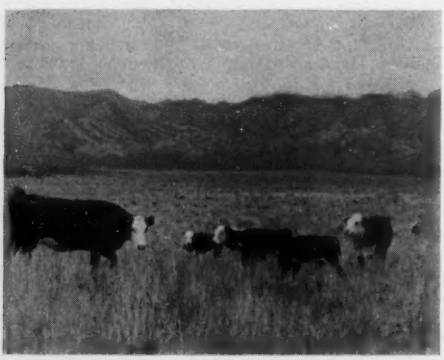

Figure 1. Cows and calves grazing seeded wheatgrasses on foothill ranges in central Utah during the spring.

were crested wheatgrass.[2] *(Agropyron cristatum)*, intermediate wheatgrass *(Agropyron intermedium)*, tall wheatgrass *(Agropyron elongatum)*, and pubescent wheatgrass *(Agropyron trichophorum)*.

The experimetnal ranges are located in west-central Utah and were formerly occupied by

Figure 2. Ewes and lambs grazing seeded foothill ranges in central Utah during the spring.

sagebrush and juniper with sparse amounts of an understory of annual weeds and a few scattered perennial grasses. Precipitation averages 12 to 14 inches per year and occurs principally as winter snows and early spring rains. The four grasses had been planted separately 3 to 10 years previous to the study in replicated, fenced pastures and were well established.

On one series of pastures containing all four wheatgrasses located at Benmore, Utah, cattle were grazed. Sheep were used on a second series of pastures near Eureka, Utah, 30 miles distant. Pubescent wheatgrass was not included at this area. Cows and calves were grazed from May 28 until July 19 during 1956 and 1957. The calves were born before the animals entered the pastures. Ewes and lambs were grazed from May 1 until July 1 during 1956 and 1957. The lambs were born in the pastures between May 1 and May 19. The animals were weighed every two weeks.

All pastures were grazed lightly so that effect of intensity of grazing on nutrient intake and gains could be kept at a minimum. The average utilization of herbage was estimated to be from 30 to 40% for all pastures at the end of the grazing trials. Digestibility coefficients were determined by the lignin-ratio technique (Cook *et al.*, 1952). Fecal

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[2] This species was a commercial purchase and contained both fairway and standard crested wheatgrass.

samples for determining chemical content were collected at both Benmore and Eureka by two individuals twice each week from each of two pastures of each species. Fresh fecal material was gathered carefully from the ground to avoid contamination from soil. Samples from each pasture for each individual collector were composited for chemical analysis.

Similarly, three times each week two individuals collected samples of forage of each species for analysis. Samples representing ingested material were hand plucked by following animals and collecting material comparable to that being eaten. Again, samples from each pasture for each individual collector were composited and analyzed chemically.

To compare livestock responses with the nutrient content of the forage, the chemical compositions of the forage samples and the calculated digestion coefficients for each species were averaged for each two-week weigh period.

Results

Nutrient Content of Cattle Diets. As the stage of plant growth advanced, total protein in the diet of cattle decreased, cellulose increased, and generally lignin increased. Other constituents showed no definite trend (table 1).

In most cases, the digestibility of the feed constituents decreased as the season advanced. This was particularly true of protein (table 2). As a result of decreasing amount and decreasing digestibility of protein, digestible protein in the diet decreased from an average of about 7% during the first period to about 2% during the last period. In most cases, metabolizable energy and total digestible nutrients decreased.

As shown by growth stage records in table 1, crested and pubescent wheatgrass matured more rapidly than intermediate and tall wheatgrass. As a result, digestible protein, phosphorus and the indexes to energy (T.D.N. and metabolizable energy) remained somewhat higher for the late maturing grasses as the season advanced (table 2).

TABLE 1. AVERAGE CHEMICAL COMPOSITION OF THE DIET OF CATTLE GRAZING FOUR SEPARATE WHEATGRASSES ON AN OVEN-DRY BASIS DURING LATE SPRING AND EARLY SUMMER OF 1956 AND 1957 AT BENMORE, UTAH

Date and species	Average state of growth	Ether extract	Total protein	Ash	Lignin	Cellulose	Other carbohydrates	Phosphorus	Gross energy
		%	%	%	%	%	%	%	Cal./lb.
May 28 to June 13									
Crested	Pre-anthesis	2.4	12.1	7.2	8.3	31.2	38.9	0.13	2010
Intermediate	Early head	3.2	12.6	7.9	6.7	29.8	39.7	.16	2009
Tall	4th leaf	2.8	11.1	9.4	7.6	31.8	37.2	.14	1978
Pubescent	Early head	2.4	10.6	7.8	7.5	31.6	40.0	.14	1959
June 14 to June 28									
Crested	Milk-dough	2.6	6.8	5.6	8.7	34.3	42.0	.14	2040
Intermediate	Anthesis	3.4	8.8	8.1	8.7	32.1	38.9	.17	1981
Tall	Pre-anthesis	3.4	8.2	9.4	8.8	33.2	37.0	.15	1969
Pubescent	Milk	3.0	6.8	8.0	8.5	35.0	38.7	.14	1997
June 29 to July 18									
Crested	Dough	2.0	4.3	6.0	8.8	38.2	40.7	.14	1990
Intermediate	Anthesis	2.6	6.7	7.5	8.3	37.4	37.5	.17	1954
Tall	Pre-anthesis	2.4	7.7	9.0	7.4	35.1	38.4	.20	1908
Pubescent	Milk	2.7	5.9	8.1	8.8	37.9	36.6	.15	1928

Cattle Gains. In the spring cows gained most (2.4 lb. per day) on pubescent wheatgrass, and least on tall wheatgrass (0.9 lb. per day). But by early summer, pubescent wheatgrass was poor, whereas tall wheatgrass was the best of all four grasses. Intermediate wheatgrass was second best of the four grasses during all three grazing periods. By summer, crested wheatgrass was the poorest grass of all.

Calves showed much less variation in gain both from different grazing seasons and from different grass species than did cows. Presumably poor feed conditions show up first in cows because cows rob their own bodies of nutrients in order to supply their calves (table 3). The uniformly high quality of intermediate wheatgrass as cattle forage was also reflected in the calf gains. On this grass,

TABLE 2. AVERAGE DIGESTIBILITY OBTAINED WITH CATTLE BY THE USE OF LIGNIN-RATIO TECHNIQUE FOR WHEATGRASSES DURING THE LATE SPRING AND EARLY SUMMER OF 1956 AND 1957, BENMORE, UTAH

Date and species	Digestibility coefficients					Digestible protein	Metabolizable energy[a]	Total digestible nutrients
	Ether extracts	Total protein	Cellulose	Available carbohydrates	Gross energy			
						%	Cal./lb.	%
May 28 to June 13								
Crested	—16.0	64.4	66.9	69.2	55.6	7.8	901	55.6
Intermediate	—18.9	62.5	74.1	74.2	58.6	7.8	872	59.5
Tall	—27.5	59.0	74.2	66.4	54.6	6.5	823	54.8
Pubescent	—47.7	56.4	68.8	67.0	51.9	6.0	794	54.5
June 14 to June 28								
Crested	—16.1	44.2	58.8	63.8	49.8	3.0	805	50.0
Intermediate	—21.9	50.3	73.2	68.6	54.0	4.4	862	54.6
Tall	4.4	50.6	73.4	63.6	53.3	4.1	869	52.4
Pubescent	—27.8	36.7	68.4	59.6	47.6	2.3	790	49.3
June 29 to July 18								
Crested	5.5	24.2	57.7	65.5	49.5	1.0	781	49.7
Intermediate	—18.7	41.2	66.4	65.5	51.1	2.8	833	52.3
Tall	—4.2	50.0	66.0	67.1	53.6	3.8	835	52.8
Pubescent	—19.4	44.9	63.1	62.3	50.6	2.6	793	49.3

[a] Loss of energy through urine in calculating metabolizable energy was taken from (Cook *et al.*, 1956).

calves gained substantially more after June 14 than on any other grass tested. For the entire grazing season, gains of both cows and calves were decidedly better on the intermediate and tall wheatgrasses.

It should be pointed out that reduced gains as the season progressed is not only a result of lower nutrient content of the forage, but may also be a result of decreased daily intake of forage because of decreased palatability due to plant maturity (Gomm, 1956).

Nutrient Content of Sheep Diets. The chemical content of the diet of ewes grazing wheatgrasses is shown in table 4. The percent total protein, phosphorus, and ash in the diet decreased as the grazing season advanced. Lignin and cellulose increased. Ether extract, other carbohydrates, and gross energy showed no definite trend (table 4).

The digestibility of each constituent tended to decrease as plants matured. However, especially during the middle of the grazing season, the digestibility of some constituents was maintained or even slightly increased (table 5). In a few cases, digestibility decreased during the second and third grazing trials but increased slightly during the last period. Much of this variability was believed to be a result

TABLE 3. AVERAGE DAILY GAIN, IN POUNDS, OF COWS AND CALVES GRAZING WHEATGRASS PASTURES DURING THE EARLY SPRING AND SUMMER OF 1956 AND 1957[a]

Species	May 28 to June 13		June 14 to June 28		June 29 to July 19		May 28 to July 19	
	Cows	Calves	Cows	Calves	Cows	Calves	Cows	Calves
Crested wheatgrass	1.2	2.5	0.6	1.1	—0.5	1.5	0.4	1.7
	(0.8)[b]	(2.3)	(...)	(...)	(...)	(1.4)	(...)	(...)
Intermediate wheatgrass	1.5	2.3	1.7	2.3	0.9	1.9	1.4	2.2
	(1.2)	(...)	(1.4)	(2.1)	(0.6)	(1.8)	(1.1)	(2.0)
Tall wheatgrass	0.9	2.6	2.0	1.9	1.8	1.5	1.6	2.0
	(...)	(2.4)	(1.7)	(1.7)	(1.5)	(1.4)	(1.3)	(1.8)
Pubescent wheatgrass	2.4	2.4	0.6	1.4	0.2	1.2	1.1	1.7
	(2.1)	(2.2)	(...)	(1.1)	(—0.1)	(...)	(0.8)	(...)

[a] Sixteen cows and 16 calves grazed in each wheatgrass treatment.
[b] Figures in parentheses are lower significant limits. Any mean having a lower value than the L.S.L. of any other mean in the column is significantly lower than that mean at the 5% level.

TABLE 4. AVERAGE CHEMICAL COMPOSITION OF SHEEP DIETS FOR THREE WHEATGRASSES ON AN OVEN-DRY BASIS DURING SPRING OF 1956 AND 1957 AT EUREKA, UTAH

Date and species	Average state of growth	Ether extract	Total protein	Ash	Lignin	Cellulose	Other carbohydrates	Phosphorus	Gross energy
		%	%	%	%	%	%	%	Cal./lb.
May 1 to May 18									
Crested	3rd leaf	2.2	14.5	12.5	6.7	26.7	37.4	0.16	1921
Intermediate	3rd leaf	2.3	16.8	14.6	6.3	23.5	36.5	.19	1875
Tall	3rd leaf	1.7	15.0	15.2	6.4	27.0	34.7	.18	1861
May 19 to June 3									
Crested	Boot	2.2	10.0	11.6	7.1	31.5	37.6	.16	1890
Intermediate	5th leaf	2.5	12.2	11.9	6.2	29.7	37.5	.17	1923
Tall	4th leaf	2.0	12.3	12.8	7.5	33.1	32.3	.17	1889
June 4 to June 17									
Crested	Head	2.0	6.6	10.3	8.5	31.0	41.6	.13	1871
Intermediate	Boot-head	3.3	11.5	10.0	6.0	29.6	39.6	.18	1961
Tall	4th-5th leaf	2.7	11.0	11.6	7.0	32.0	35.7	.12	1944
June 18 to July 1									
Crested	Hard dough	2.9	5.9	8.6	8.8	33.7	40.1	.10	1904
Intermediate	Anthesis	3.5	10.1	9.4	6.4	30.6	40.0	.16	1954
Tall	Early head	3.1	10.7	10.6	7.8	32.7	35.1	.11	1933

of a change in portions of the plants consumed from time to time during the grazing trials. Sheep were more selective of the various parts of the plant than cattle. Sheep ate more leafy material and avoided dry and coarse portions of the plant.

The digestible protein in sheep diets, like cattle diets, decreased drastically as a result of gradual decrease in protein in the diet and decreased digestibility as the grazing season progressed (table 5).

The metabolizable energy for crested wheatgrass decreased markedly from the first of May until mid June; whereas, the energy content of intermediate wheatgrass increased decidedly during this same period. The metabolizable energy of tall wheatgrass followed no trend.

TABLE 5. AVERAGE DIGESTIBILITY OBTAINED FOR SHEEP BY THE USE OF LIGNIN-RATIO TECHNIQUE FOR WHEATGRASSES DURING THE SPRING OF 1956 AND 1957 AT EUREKA, UTAH

Date and species	Digestibility coefficients					Digestible protein	Metabolizable energy[a]	Total digestible nutrients
	Ether extracts	Total protein	Cellulose	Available carbohydrates	Gross energy			
						%	Cal./lb.	%
May 1 to May 18								
Crested	—25.0	55.4	71.1	73.9	58.5	8.0	943	54.8
Intermediate	—30.6	59.5	72.2	73.2	56.4	10.0	783	53.4
Tall	—18.2	61.2	73.8	71.4	59.3	9.2	839	53.9
May 19 to June 3								
Crested	—0.4	50.0	71.1	67.8	55.2	5.0	718	52.9
Intermediate	7.1	56.3	78.9	76.1	62.4	6.3	890	58.7
Tall	—37.4	52.9	76.9	62.3	54.2	6.5	779	52.1
June 4 to June 17								
Crested	—36.0	27.8	64.3	52.0	48.0	1.8	621	43.4
Intermediate	23.4	63.7	79.6	75.2	64.4	7.3	958	62.4
Tall	1.8	65.8	75.1	69.1	59.2	7.2	880	56.0
June 18 to July 1								
Crested	—20.0	25.2	57.3	66.4	50.6	1.5	638	47.4
Intermediate	20.2	50.9	74.2	73.3	64.1	5.1	904	58.7
Tall	—12.4	60.1	71.1	66.6	55.3	6.4	824	53.0

[a] Losses of energy through urine in calculating metabolizable energy was taken from (Cook *et al.*, 1956).

Total digestible nutrients followed the same general trends as metabolizable energy for all three species (table 5).

Sheep Gains. Both ewes and lambs gained more throughout the grazing season on intermediate wheatgrass than on tall or crested wheatgrass (table 6).

Lactating ewes lost weight during all spring grazing periods on both crested and tall wheatgrass pastures; whereas they gained on intermediate wheatgrass during all periods (table 6). Weight losses on both crested and tall wheatgrass pastures were heaviest in late season.

of tall wheatgrass was higher because cattle preferred tall wheatgrass late in the season.

Discussion

Crested and pubescent wheatgrasses grow in the early spring and mature early. Crested wheatgrass is particularly early growing and a good pasture of this species can be grazed at least a week earlier than native sagebrush-grass ranges. In contrast, intermediate and tall wheatgrasses start growth later and are substantially later in their development and maturity.

In central Utah, crested and pubescent

TABLE 6. AVERAGE DAILY GAIN, IN POUNDS, OF EWES AND LAMBS GRAZING WHEATGRASS PASTURES DURING THE SPRINGS OF 1956 AND 1957 [a]

	May 1 to May 18		May 19 to June 3		June 4 to June 17		June 18 to July 1		May 1 to July 1	
Species	Ewes	Lambs	Ewes [b]	Lambs	Ewes	Lambs	Ewes	Lambs	Ewes	Lambs
Crested wheatgrass	0.47 (0.28) [c]	....	—0.04 (—0.20)	0.52 (....)	—0.18 (....)	0.52 (0.49)	—0.29 (—0.40)	0.34 (....)	—0.01 (—0.23)	0.46 (....)
Intermediate wheatgrass	0.41 (0.21)	....	0.17 (0.02)	0.66 (0.61)	0.16 (0.04)	0.55 (0.52)	0.32 (0.22)	0.52 (0.47)	0.26 (0.06)	0.58 (0.52)
Tall wheatgrass	0.36 (....)	....	—0.16 (....)	0.55 (0.49)	—0.04 (—0.17)	0.47 (....)	—0.65 (....)	0.37 (0.31)	—0.12 (....)	0.46 (....)

[a] One hundred and forty ewes grazed each wheatgrass treatment, 169 lambs in crested wheatgrass and 171 in each of the intermediate and tall wheatgrass treatments.

[b] Fifteen pounds was added to each ewe weight for the May 19 to June 3 period to compensate for the loss in weight resulting from birth of the lamb.

[c] Figures in parentheses are lower significant limits. Any mean having a lower value than the L.S.L. of any other mean value in the column is significantly lower than that mean at the 5% level.

Lamb gains, like ewe gains, were somewhat less during the late spring. Ewes, like cows, sacrifice body weight when necessary to maintain milk flow for lamb welfare. Therefore, low nutrient intake may be reflected in ewe weights long before it is evident in lamb weights.

Grazing Grass Mixtures. At both experimental areas, animals were grazed also on pastures supporting approximately equal amounts of the same grasses planted together in a mixture. Both sheep and cattle made gains approaching their averages on these grasses grazed separately. However, after 3 to 4 years of grazing, the highly palatable intermediate wheatgrass in the mixed stand had been killed out to an alarming extent. This was true for both cattle grazing and sheep grazing. Tall wheatgrass, likewise, had decreased in the mixtures at both areas. However, at Eureka this was probably natural loss as a result of a 1956 summer drought, since pure stands of tall wheatgrass were also reduced correspondingly. At Benmore the loss

wheatgrasses should be used during early spring or until about June 8 for greatest effectiveness. Following this, animals can be grazed on intermediate or tall wheatgrass. However, in the case of sheep, tall wheatgrass is not nearly as suitable as intermediate wheatgrass for late spring grazing. For lactating cows, tall wheatgrass is suitable for grazing as late as midsummer if not too heavily grazed or if the summer is not too dry. Actually, cattle utilize both crested and tall wheatgrasses better than sheep, since at all times in the spring, lactating cows actually gained weight on these species, whereas lactating ewes lost weight during all grazing periods.

For efficiency in use, it is evident that pure stands of these wheatgrasses are preferable to mixtures. When plants of different palatability are planted together, the manager is faced with either overgrazing the more palatable or with wasteful underuse of the less palatable species.

There appear to be two alternatives in correcting a deficiency of spring range. First, livestock men can seed some of their foothill

ranges to an early-growing grass such as crested or pubescent wheatgrass for early spring grazing, and other areas can be seeded to a late-maturing grass such as intermediate or tall wheatgrass for late spring grazing. Second, if native sagebrush-grass range is available, it will satisfy the need for moderately early spring grazing. However, low-elevation native ranges usually are not suitable for late spring grazing and livestock should be moved either to seeded grass pastures more suitable for late grazing or to native mountain brush ranges at higher elevations. It is doubtful whether dry wheatgrass pasturage is suitable for either cattle or sheep during summer and fall under central Utah weather conditions.

Summary

During the spring and early summer of 1956 and 1957 a study was conducted in central Utah to determine nutritive intakes and livestock gains on seeded foothill ranges.

Four wheatgrasses were studied, crested, intermediate, tall, and pubescent. Pure stands were grazed by both cattle and sheep.

In most cases, total protein, phosphorus, and energy content of the diets decreased as the grasses matured. Lignin and cellulose increased. In general, the digestibility of all constituents decreased.

Sheep and cattle made better gains early in the season than later. Late season gains by cattle were greater on the later maturing intermediate and tall wheatgrasses than on earlier maturing crested and pubescent wheatgrasses. Intermediate and tall wheatgrasses also gave best season-long cattle gains. Intermediate wheatgrass gave better gains throughout the grazing season for both ewes and lambs than did tall or crested wheatgrass.

It was concluded that cattle make better use of wheatgrasses than do sheep since lactating cows generally held their body weight or gained throughout spring and early summer, whereas ewes, except those grazing intermediate wheatgrass, lost weight throughout the season. Reductions in lamb and calf gains were less with increasing forage maturity than were reductions in gains of their mothers. Gains of calves and lambs were more nearly the same on different grass species than were gains of the mothers.

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EFFECT OF PELLETING STEER-FATTENING RATIONS OF DIFFERENT CONCENTRATE TO ROUGHAGE RATIOS

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PELLETING high roughage rations has been shown to increase rate of gain, feed intake and feed efficiency of cattle and sheep in numerous experiments (Webb *et al.*, 1957; Meyer *et al.*, 1959; Weir *et al.*, 1959; Wallace and Hubbert, 1959). In contrast, several studies have indicated little or no improvement in rate of gain and feed intake by pelleting high concentrate rations (Menzies *et al.*, 1957; Perry *et al.*, 1959; Beardsley *et al.*, 1959; Hartman *et al.*, 1959). Digestibility trials with cattle and sheep have shown little effect of pelleting high roughage rations on apparent digestibility of the various nutrients (Esplin *et al.*, 1957; Lindahl and Reynolds, 1959; Meyer *et al.*, 1959). Although carcass data from cattle fed pelleted rations are somewhat limited, a general improvement in yield and grade has been reported by some workers (Weir *et al.*, 1959; Perry *et al.*, 1959).

This experiment was conducted to determine the effects of pelleting complete, mixed rations of widely different concentrate to roughage ratios on the feedlot performance and carcass merit of fattening steers and on the digestibility of nutrients in those types of rations.

Experimental Procedure

In two fattening trials, each involving 24 steers, rations with 1:4 and 4:1 ratios of concentrate to roughage were fed in unpelleted and pelleted forms. Steer calves initially weighing about 490 lb. were used in the first trial and yearling steers weighing 630 lb. in the second trial. Similar rations were fed in each trial. The concentrate was composed of ground milo, cottonseed meal and molasses; equal parts of alfalfa hay, chopped to ¾-inch lengths, and cottonseed hulls comprised the roughage (table 1). Calcium, phosphorus and digestible protein contents of the rations were approximately equal according to Morrison's tables (1956). The pelleted ration was fed as 3/8-inch pellets; the unpelleted ration was fed in the loose form.

In the first trial, the steer calves were selected from the experiment station herd at weaning and assigned to treatment according to shrunk weight, feeder grade, sire and age of dam. In the second trial, a uniform group of yearling steers was selected from a large commercial herd and allotted to treatment on the basis of shrunk weight and feeder grade. Initial weights were taken after the cattle were on full feed. The cattle in both trials were self-fed in individual pens (10 feet × 40 feet) with free-choice access to water and a mineral mixture (2 parts salt and 1 part steamed bonemeal). Initial and final weights were taken following an 18-hour period off feed and water.

The steers were fed 176 days and 148 days in Trials I and II, respectively. Intermediate weights were taken at 28-day intervals and individual records maintained on rate of gain, feed consumption and feed efficiency. At slaughter, data were collected on dressing percent and carcass grade. The wholesale rib cut from the right side of each carcass was removed and composition of the 9–10–11th rib cut determined by physical separation for

TABLE 1. COMPOSITION OF STEER RATIONS

Concentrate: roughage ratio	1:4		4:1	
Ingredients, %				
Ground milo	1.0		65.1	
Cottonseed meal	12.0		7.0	
Molasses	7.0		7.0	
Cottonseed hulls	40.0		10.0	
Chopped alfalfa	40.0		10.0	
Ground limestone	....		0.9	
Proximate composition, %[a]	Unpelleted	Pelleted	Unpelleted	Pelleted
Dry matter	90.67	90.33	88.93	89.86
Ash	9.26	8.83	5.14	5.12
Crude protein	15.48	15.23	13.51	13.12
Ether extract	1.95	2.04	3.05	2.98
Crude fiber	33.96	32.70	10.96	11.29
N-free extract	39.35	41.20	67.34	67.49

[a] Analysis made on rations used in Trial II.

estimation of carcass composition, using the technique of Hankins and Howe (1946). A composite sample from the *Longissimus dorsi* muscle of each rib cut was analyzed for water, ash, fat and protein.

Digestibility of the rations was determined during Trial II by the chromic oxide method. A premix of 10 parts cottonseed meal, 2 parts chromic oxide, 1 part molasses and 1 part water by weight was prepared. This premix was mixed uniformly with the ration in a 1000-lb. capacity mixer to give a concentration of 0.2% chromic oxide in the ration. The cottonseed meal content of the rations was adjusted so that the ration composition was essentially the same after the premix was added. The ration containing the indicator was fed for a nine-day preliminary period and a five-day collection period. Grab samples were taken twice daily (8 a.m. and 4 p.m.) for 5 days and composited for each steer. Chemical analyses of feed and feces were made and digestibility values calculated for dry matter, protein, crude fiber, ether extract and nitrogen-free extract.

Results and Discussion

Rate of Gain. Average results of the feedlot and carcass data for each trial are shown in table 2. The trials are reported separately since the response to pelleting the high concentrate ration appeared to differ slightly between trials. In both trials, steers receiving the 1:4 (concentrate: roughage) pelleted ration gained significantly faster ($P<.01$) than those fed the 1:4 unpelleted ration. An average of the two trials shows that the cattle fed the 1:4 pelleted ration outgained those fed the unpelleted ration by 21%. In contrast, cattle fed the 4:1 pelleted ration gained less than those fed the unpelleted ration in Trial I but gains on the two forms were about equal in Trial II. The different response to pelleting the 4:1 ratio in Trials I and II may have been influenced by age of cattle and season.

Some workers, Jensen *et al.* (1958) and Beardsley *et al.* (1959), have observed rumen parakeratosis in lambs and cattle fed pelleted fattening rations, which may have influenced rate of gain. No incidence of rumen abnormalities was observed in the present trials.

Feed Consumption and Efficiency. Daily feed consumption of steers fed the pelleted 1:4 ration was greater than that of those fed the unpelleted ration, but the difference was significant ($P<.01$) only in the second trial. An average of the two trials shows that feed intake on the 1:4 ration was 11% greater for the pelleted than for the unpelleted ration.

TABLE 2. RESULTS OF FEEDING TRIALS AND CARCASS STUDIES

	Trial I				Trial II			
Concentrate:roughage ratio	1:4		4:1		1:4		4:1	
Form of ration	Unpelleted	Pelleted	Unpelleted	Pelleted	Unpelleted	Pelleted	Unpelleted	Pelleted
No. of steers	6	6	6	6	6	6	6	6
Days on feed	176	176	176	176	148	148	148	148
Initial weight	488	482	503	509	634	629	622	623
Final weight	813	885	893	844	912	967	977	986
Daily gains, lb.	1.85	2.29**	2.21	1.90	1.88	2.28**	2.40	2.45
Daily feed intake, lb.	19.7	22.5	18.7	16.2	26.7	31.6**	25.3	23.0
Feed/lb. gain, lb.	10.7	9.9	8.5	8.4	14.2	13.9	10.6	9.4
Carcass data								
Dressing, %	59.4	58.8	61.7	60.3	59.4	59.9	61.8	63.1
Grade[a]	7.7	7.3	5.8	7.3	7.1	6.8	4.8	5.8
Composition of rib cut, %								
Lean	53.0	53.1	51.5	54.6	46.5	43.9	43.5	43.5
Fat	31.1	32.1	33.7	30.3	38.7	42.0	43.7	44.1
Bone	15.6	15.0	14.4	15.0	14.6	13.8	12.5	12.0
Composition of *L. dorsi*, %								
Water	73.46	73.42	73.09	73.68	72.52	71.65	70.45	71.48
Ash	1.15	1.13	1.12	1.20	1.04	1.03	1.07	1.04
Fat	3.18	3.31	4.30	3.76	4.23	4.93	6.24	5.80
Protein	21.99	21.85	21.77	21.72	21.43	21.36	21.27	21.33

** Indicates significance at the 1% level of probability.
[a] 5=Low choice, 6=High good, 7=Average good, 8=Low good.

Pelleting the 4:1 mixture resulted in a reduction in feed intake in both trials; however, differences were not statistically significant.

Feed efficiency in both trials was greater when the rations were fed in the pelleted form. An average of the two trials shows that pelleting resulted in 5.6% and 7.5% less feed per pound of gain for the 1:4 and 4:1 rations, respectively. Since the increase in feed efficiency from pelleting the 1:4 ration was very small compared to the increase in rate of gain, it would seem that increased feed consumption was largely responsible for the improvement in gain. In a similar study with lambs, Meyer *et al.* (1959) concluded that the increase in gain resulting from feeding finely ground and pelleted alfalfa hay was due to an increase in feed intake which apparently resulted from a faster rate of passage of ingesta through the reticulo-rumen. Balch (1950) found that small particles are removed from the reticulo-rumen faster than large ones. Esplin *et al.* (1957) found that gains and efficiency of feed conversion of lambs were the same for pelleted and unpelleted rations when feed intake was equalized. Blaxter and Graham (1956) concluded that physical factors such as fine grinding and cubing which change the rate of food passage may also change the rate and nature of microbial fermentation.

Although both the meal and pelleted rations were originally of the same particle size a certain amount of physical breakdown probably occurred during the pelleting process, resulting in finer particle size in the pelleted rations. This may have influenced rate of passage of ingesta through the reticulo-rumen when the 1:4 ratio was fed as pellets.

Carcass Data. The carcass data shown in table 2 indicate no significant change in dressing percent due to pelleting either ration. Steers fed either form of the 4:1 ration were significantly higher ($P<.01$) in dressing percent than those fed the 1:4 ration. Similarly, carcass grades of cattle on the 4:1 ration were higher than those on the 1:4 ration. There was no appreciable difference between carcass grades of steers fed the two forms of the 1:4 ration; however, in both trials, cattle fed the pelleted 4:1 ration graded approximately ⅓ grade lower than those on the 4:1 unpelleted ration. These results are similar to those obtained by Beardsley *et al.* (1959). However, Perry *et al.* (1959) found that pelleting high roughage rations for steers resulted in an increase in grade and dressing percent, whereas pelleting a high concentrate ration had little effect on grade and yield.

Physical separation of the 9–10–11th rib cuts revealed no appreciable effect of concentrate to roughage ratio on percent lean, fat or bone in the rib cuts in Trial I; however, in Trial II ribs from cattle fed the 4:1 rations contained 3.6% more fat, 1.7% less lean and 1.9% less bone than those from cattle on the 1:4 rations. Chemical analysis of a composite sample from the *Longissimus dorsi* muscle of the 9–10–11th rib cut showed no difference between ratios in either trial in the percent of water, ash or protein; however, an average of the two trials shows that the samples from steers on the 4:1 ration contained 1.1% more ether extract than those on the 1:4 ration.

TABLE 3. APPARENT DIGESTIBILITY OF NUTRIENTS IN RATIONS (TRIAL II)

Concentrate: roughage ratio	1:4		4:1	
Form of ration	Unpelleted	Pelleted	Unpelleted	Pelleted
Dry matter	47.0	46.7	54.8	57.5
Protein	53.4	49.8	46.4	45.6
Fat	75.5	84.2	87.3	76.3
Fiber	48.8	43.4	19.7	10.6
N.F.E.	53.6	57.9	70.1	75.3

Pelleting produced no significant differences in percent separable lean, fat and bone of the rib cuts, or chemical composition of the *Longissimus dorsi* sample from each cut. However, in both trials, pelleting the 1:4 ration tended to increase the percent of fat in the rib cuts and *Longissimus dorsi* samples. Similarly, Weir *et al.* (1959) observed a significant increase in carcass fat when a 100% alfalfa ration was pelleted, and a significant decrease when a 60% concentrate mixture was pelleted.

Digestibility Study. Results of the digestibility study are shown in table 3. Differences between the unpelleted and pelleted rations in apparent digestibility were not statistically significant. There was a trend toward slightly lower digestibility of crude fiber when either ration was fed in the pelleted form. This agrees with results obtained by Wallace and Hubbert (1959) and Weir *et al.* (1959). Average fat digestibility was improved slightly by pelleting the 1:4 ration but was reduced by pelleting the 4:1 ration. Digestibility values for crude fiber on both forms of the 4:1 ration were low. The reason for these low values is not

apparent, but they may be due in part to a rapid rate of food passage from the reticulorumen, allowing only a small amount of microbial breakdown of the fibrous portion of the ration. Similar values for crude fiber digestibility were observed by Bloom *et al.* (1957) with dairy cows fed four ratios of concentrate to roughage. Putnam and Loosli (1959) found that as the proportion of concentrate increased in a dairy cow ration the apparent digestibility values increased for dry matter, crude protein, ether extract, nitrogen-free extract and total carbohydrates. However, digestibility of crude fiber decreased as the proportion of concentrate increased. Andersen *et al.* (1959) found that nutrient digestibility of steer rations decreased as the level of ration consumption increased. These workers observed that steers may derive 8 to 25% less digestible matter from high concentrate rations consumed at production levels of feed intake than from the same rations at the maintenance level.

Thus, the low crude fiber digestibility values apparently resulted from feeding the high concentrate ration at a high level of intake. Also small errors in fiber analysis of feed and feces are proportionately greater on the high-concentrate ration due to the small amount of fiber in the ration. Results of this trial indicate that changes in nutrient digestibility due to pelleting the two rations did not account for much of the differences in rate of gain.

Summary

Forty-eight Hereford steers were individually self-fed in two trials to study the effects of pelleting rations with 1:4 and 4:1 concentrate to roughage ratios on feedlot performance and carcass merit. Rate of gain and feed intake were significantly increased ($P<.01$) by pelleting the 1:4 ration. Pelleting the 4:1 ration resulted in no significant change in rate of gain but caused a decrease in feed intake. Feed efficiency on both rations was improved slightly by pelleting. Dressing percent was unaffected by pelleting; carcass grades were not affected by pelleting the 1:4 ration but were reduced slightly by pelleting the 4:1 ration. Chemical or physical composition of the 9–10–11th rib cuts was not significantly affected by form of the two rations. Apparent digestibility of ration components was not significantly altered by pelleting either ration, although it did tend to reduce digestibility of crude fiber.

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INFLUENCE OF STOCKING RATE ON ANIMAL AND FORAGE PRODUCTION FROM IRRIGATED PASTURE

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GRAZING intensity or stocking rate, one of the most easily controlled factors in pasture management, has received little attention in grazing research. Yet, McMeekan (1956), in his review of research from Ruakura, reports that increased stocking rate of dairy cows and sheep resulted in greater production per acre even though gains and production per animal decreased. Hancock (1958) reports later results which are in agreement. Similarly, Frischknecht *et al.* (1953), with beef steers grazing on range forage, reported comparable results. That stocking rate can distort experimental results has also been suggested by McMeekan (1956) and later by Freer (1959). These workers have reported no difference between strip and rotational grazing with dairy cows when there was no difference in stocking rate. This contrasts with the results of other workers (Holmes *et al.*, 1950, 1952; Proctor and Hood, 1953) where greater production resulted from strip grazing than from rotational grazing; stocking rate, however, was greater for the strip grazing treatment. Meyer *et al.* (1956) found no difference between these two systems of grazing management with beef steers but had suggested that length of rotational grazing interval might have an influence.

Recently Pieper *et al.* (1959) reviewed the literature and reported some of their own results which revealed that increased grazing intensity resulted in a decreased digestible nutrient content of the consumed forage. That this occurs was not surprising because increased numbers per acre forces animals to eat the less digestible and less palatable forage to obtain sufficient intake. McMeekan (1956), Freer (1959) and Frischknecht *et al.* (1953) recognized this as the reason for increased production when stocking rate was increased; consumption of more of the available forage, though possibly less digestible and palatable, was forced upon animals by increased stocking rate. No doubt a diminishing return would be realized if stocking rate was increased to such an extent that reduced feed intake of low-quality forage would result in little or no production. A study has not been made to show how critical this point is in relation to stocking rate and increased or decreased production per acre.

To be reported in this paper is a study on the influence and importance of widely varying stocking rates on both forage and animal production from irrigated pasture. Increased precision for the comparisons was obtained by adding body composition of the steers and intake and digestibility of the forage to the usual measurements of weight gains, clipping yields and chemical composition of the forage.

Experimental

A uniform field of orchardgrass *(Dactylis glomerata)* and Ladino clover *(Trifolium repens)* was divided into 36 pastures of 0.37 acre each. These were divided into six blocks of six pastures each—one pasture within a block for each treatment. The pastures within a block were grazed for 7 days; the animals were then moved to the next block where each pasture was grazed for 7 days. This gave each pasture 7 days' grazing followed by a 35-day recovery period for the forage before it was grazed again. Three grazings were given each pasture, resulting in 126 days for the experiment. The first five treatments consisted of 3, 5, 7, 9 and 11 steers, respectively, for each pasture (0.37 acre each), resulting in a stocking rate of 1.35, 2.25, 3.06, 3.93 and 4.68 steers per acre (table 1). Soilage harvested from the sixth pasture was given to a lot of eight steers. This served as a positive control because the response from this treatment should be similar to what might be expected from pasturing forage without the superimposed animal influence.

Management of the forage included adequate irrigation and fertilization, 30 units of nitrogen applied per acre per month. In the

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TABLE 1. COMPOSITION OF FORAGE (DRY BASIS)

Item	Pasture					Soilage
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
Animals grazed per acre	1.35	2.25	3.06	3.93	4.68	3.24
Hand-clipped samples:						
Legumes, %	42	46	46	52	47	44
Lignin, %	5.2[a]	4.9[a]	4.8[a]	4.4[a, b]	4.2[b]	4.9[a]
Crude protein, %	19	19	20	20	21	20
Forage consumed:						
Digestible energy, mcal./100 lb.	130[a]	132[a]	126[b]	128[b]	122[c]	126[b]

[a] Values with the same letter superscript are not significantly different. Values with different letter superscripts are significantly different (P=0.05).

spring before the experiment began, the forage was harvested by a field chopper the proper number of days before the start of the experiment to establish the same recovery interval (35 days) for each block. The forage thus removed was not credited to the experiment.

Hereford feeder steers were obtained 30 days before the experiment began so that 18 steers (3 per treatment) could be gentled and accustomed to fecal collections. All animals were allotted at random. Initial weights were not taken until the animals had been on pasture for 6 days. All weights were taken after 14 hours without feed and water. Initially seven steers were selected at random, slaughtered, and body composition estimated from carcass specific gravity. The methods suggested by Meyer *et al.* (1960) were applied to the data.

Available forage was measured by the clipping technique. Before the steers entered a pasture, 5 to 10 areas of 18 sq. ft. each were clipped at random, 2 inches high. Dry matter and species determinations were made. Samples were saved and composited for chemical analysis.

Digestibility and feed intake were measured by the chromogen-chromium oxide technique of Reid *et al.* (1952). Digestibility and forage consumption were measured four times during the trial. Twenty grams of chromium oxide were administered daily at 6 a.m. during a preliminary period of 7 days. Grab fecal samples were then collected twice daily from the rectum of the collection steers at 6 a.m. and 4 p.m. during the next 7 days. All results are on a silica-free basis because of dust contamination of the forage. The method of Kimura and Miller (1957) was used for the chromium oxide determination. TDN was calculated as described by Lofgreen and Meyer (1956) and converted to digestible energy by a conversion factor of 2,000 kcal. per pound of TDN.

Results and Discussion

That stocking rate has an influence on forage production was apparent in this experiment (figure 1). No difference was found in the amount of forage available to the animals before the pasture was first grazed. Treatment effects were not present here because the pastures had not been grazed. The forage available for second grazing, however, showed treatment effects from the first grazing and by the third grazing the treatment effects were marked. Heavier stocking rates caused statistically significant decreases in forage production as the season progressed. Harvesting with a machine (for the soilage group) was less harmful to the forage than grazing with animals—another advantage where soilage is applicable. This may also indicate that information from clipping data in small plot work with forages cannot be applied to pasturing except with extreme caution.

Even though heavy grazing had decreased the yield of forage as the 1959 season progressed, the first cutting in 1960 showed no effect of the previous year's treatment. Admittedly more data are needed, but this does cast some doubt on the fear that heavy grazing would harm these well-established forage plants. It may be that the recovery interval of 35 days was sufficient to maintain the vigor of the plant, even though heavy grazing reduced the number of growing points per clover plant. Furthermore, the more intense grazing removed forage from below the height cut by the harvester, and these plants had more distance to grow. Weir *et al.* (1960) reported that alfalfa cut at an immature growth stage for 3 years, even though showing decreased yields compared to alfalfa cut at more mature stages, recovered completely and produced as well the fourth year when all were harvested at 1/10th bloom. Furthermore, Frischknecht

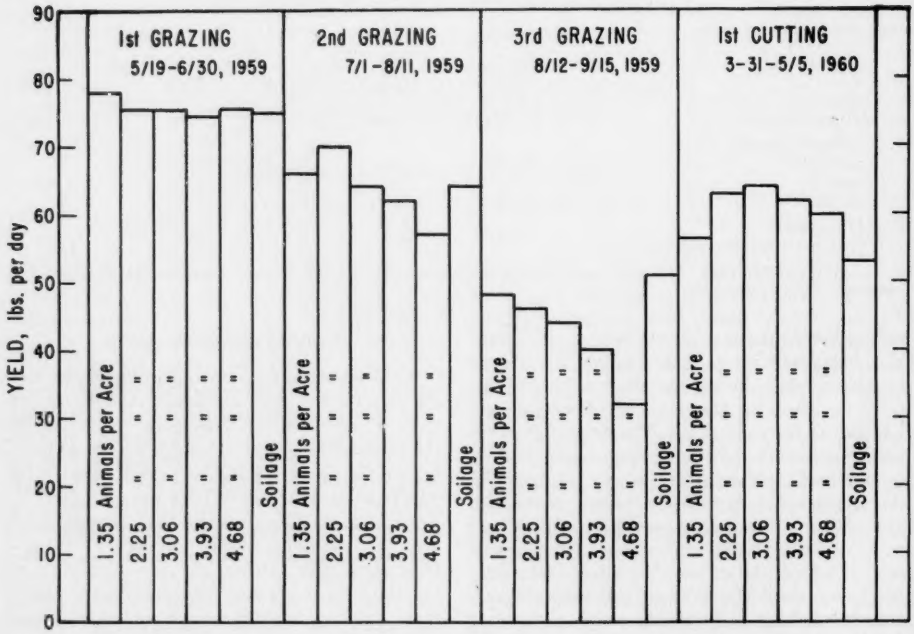

Figure 1. Forage produced per acre daily as determined by pre-grazing clipping at a height of 2 inches.

et al. (1953), in a four-year study with crested wheatgrass, could find little difference between the effects of light, moderate, and heavy grazing.

Intensity of grazing influenced the chemical composition of the clipped forage (table 1), resulting in a more nutritious forage when more heavily grazed. Lignin decreased and protein increased in the available forage as stocking rate increased. The forage was probably less mature because of a slower recovery as indicated by the decreased yield of the more heavily grazed pastures (figure 1). As indicated by the digestible energy, the forage consumed by the steers, as contrasted to that available, decreased in quality as the stocking rate increased. Nevertheless, this contrast can be explained because heavier stocking rates force animals to consume more of the coarser portions of the forage even though the whole plant was lower in lignin. The steers on the more lightly grazed pastures could select a more nutritious diet.

The digestible energy content of the forage consumed by the soilage steers, 126 mcal., per 100 lb., might be said to represent the energy value of the standing forage before grazing. A light stocking rate allowed the steers freedom to select a more nutritious forage, from 130 to 132 mcal. per 100 lb., whereas heavy stocking forced the steers to consume a less nutritious forage, 122 mcal. per 100 lb. Two conclusions can be drawn from these data: first, chemical analysis of pregrazed forage was not indicative of the quality of forage consumed, and second, stocking rate has a real influence on the quality of forage *consumed* by the steers.

The response of the steers was markedly influenced by stocking rate (table 2). As grazing intensity increased from 1.35 to 4.68 steers per acre, feed and energy intake decreased, weight gain decreased and the energy content of the carcass decreased. The corrected carcass weight adjusted in the analysis of covariance to equal initial weight which should be the measure of preference,[2] significantly decreased with each increase in stocking rate. The soilage lot responded, for most measurements, in a manner similar to lots 2 and 3.

Figure 2 depicts, for the 126-day experi-

[2] From the known fat content, calculated by specific gravity, the carcass weight is adjusted to one which has an energy value of 1,297 kcal. per lb. (Meyer *et al.*, 1960).

TABLE 2. STEER RESPONSE TO TREATMENTS[1]

Item	Pasture					Soilage
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
Animals grazed per acre	1.35	2.25	3.06	3.93	4.68	3.24
No. of steers per lot	3	5	7	9	11	8
Av. daily gain, lb.	1.81	1.72	1.44	1.31	0.80	1.49
Final weight, lb.	842[a]	832[a, b]	796[c]	780[c]	715	802[b, c]
Daily forage intake, lb.	16.9	14.5	13.8	12.7	13.2	18.0
Gain/100 lb. feed, lb.	10.7	11.9	10.4	10.3	6.1	8.3
Digestible energy intake, mcal.	22.0[a]	19.0[a, b]	17.1[b, c]	16.0[c]	16.3[c]	22.8[a]
Carcass data:						
Weight, lb.	489[a]	471[a]	459[a]	433	415	466[a]
Fat, %	17.1[a]	14.7[b, c]	14.8[b, c]	13.9[b]	13.4[b]	16.5[a, c]
Protein, %	18.9	19.1	19.1	19.1	19.2	19.0
Energy, mcal./lb.	1214[a]	1119[b]	1122[b]	1085[c]	1063[c]	1192[a]
Corrected carcass wt., lb.[2]	452[a]	424[a, b]	393[b, c]	367[c]	329	429[a, b]

[1] All weight data are corrected to an equal initial weight, 614 lb., in an analysis of covariance. The experimental period was 126 days. Forage data are on an oven-dry basis.
[2] Corrected to a carcass weight equivalent to a carcass containing 1,297 kcal. per lb., 17.3% protein and 20% fat (Meyer *et al.*, 1960).
[a] Values with the same letter superscript are not significantly different. Values with different letter superscripts are significantly different (P=0.05).

ment, the consumption of forage and digestible energy and the production per animal plotted against stocking rate. Consumption of forage and digestible energy decreased rapidly when stocking rate was initially increased but tended to level at the higher stocking rates. At this point both liveweight gain and corrected carcass gain continued to decrease. Note that the corrected carcass rather than liveweight gain (a term of uncertain composition) produced a more uniform response when expressing the results in terms of equal composition for all lots.

Figure 2. Influence of stocking rate on animal response.

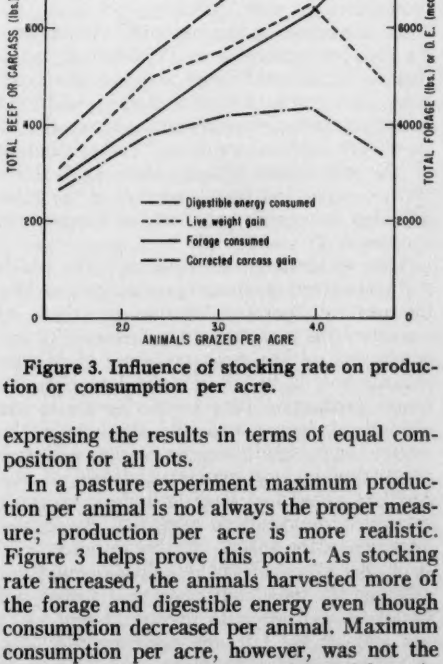

Figure 3. Influence of stocking rate on production or consumption per acre.

In a pasture experiment maximum production per animal is not always the proper measure; production per acre is more realistic. Figure 3 helps prove this point. As stocking rate increased, the animals harvested more of the forage and digestible energy even though consumption decreased per animal. Maximum consumption per acre, however, was not the most desirable in this case. Liveweight gain

and corrected carcass production per acre increased to a point—about four animals per acre—and then rapidly decreased. A most important animal-plant interaction, therefore, was present when this point was reached. Even though increased stocking rate raised consumption of forage per acre, consumption per animal was lowered sufficiently so that too much forage was used for maintenance of the animal rather than for gain. Lot 4, 3.93 steers per acre, and lot 3, 3.06, used 69% and 75%, respectively, of their digestible energy intake for maintenance, whereas lot 5, 4.68 steers per acre, used 80% for maintenance. It would appear, therefore, that maximum production per acre in this experiment was realized when not more than 75% of the digestible energy intake was used for maintenance. This difference was not great but seemingly important.

A pasture operator would not know the feed intake of his steers but would be interested in knowing the point where feed intake per animal decreases and negates the advantage of having a larger proportion of the forage consumed per acre. Weight gain might provide a guide. In this experiment, increasing the stocking rate to the point where daily gains did not go below 1.31 lb. provided maximum production per acre.

An inspection of the corrected carcass production per acre (figure 3) shows a broad plateau between 2.25 and about 4 steers per acre. The reason for this plateau was that forage consumption per acre was lower at 2.25 steers per acre but gains and energy content of the gains were higher; while at 4 steers per acre gains and energy content of the gains were low but greater amounts of forage were consumed.

This wide range of stocking rate which still gives about maximum production provides the pasture operator latitude in which to maneuver his management practices. For example, one of the main problems of pasture management is to adjust animal numbers to forage production because of changes in the amount of forage available throughout the season—high production in spring and low production in late summer and fall. If one could be assured of some latitude in stocking rate, pastures could be grazed at the lighter intensity in the spring (in this instance, 2.25 steers per acre). Here the animals would not consume the maximum amount of forage but would compensate with greater energy gains per animal. By fall, the same number that would be considered a light stocking rate in the spring would be heavy (in this case, 4 steers per acre). Here more of the available forage would be consumed but counterbalanced by lower energy gains per animal. Nonetheless, about the same production of usable energy gain per acre (corrected carcass) would be realized throughout the season.

The relationship of forage available per animal (determined by clipping) and forage consumption as measured by the chromogen-chromium oxide technique was studied. Lofgreen *et al.* (1956) and Peterson *et al.* (1956) previously reported that the clipping technique did not accurately measure the feed intake of grazing animals. Relative comparisons of different grazing systems were still possible, however. Even though we feel that pre- and post-grazing clipping will not accurately measure forage consumption by grazing animals, pre-grazing clippings should have value in estimating the proper stocking rate. Because of the great variation in stocking rate in this experiment, and hence variation in available forage per steer, it was possible to study the relationship between available forage and digestible energy consumed. Figure 4 illustrates this relationship.

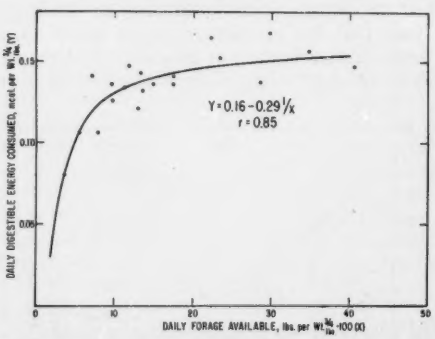

Figure 4. Relationship of consumption of digestible energy and amount of forage available to each steer.

A very high correlation (0.85) was found between the amount of forage available per steer before grazing and the amount of digestible energy consumed. This relationship indicates that small quantities of available forage prevent the steer from eating to appetite, and as the quantity of available forage increases, consumption also rises, but only to a point because energy intake would not rise above feed capacity no matter how much forage is available. Other investigators (Burger

and Foster, 1953; Schultz *et al.*, 1959; Austenson *et al.*, 1959) have shown quite good relationships between production of beef or milk per acre and available forage. Davis and Bell (1958) report no correlation between lamb gain per acre and dry matter production, but from their design this is not unexpected. They used "tester" animals for measurements and "put-and-take" animals to obtain complete forage utilization. This results in a narrow range of available forage per animal which would not be great enough for a test of this premise.

Aside from the main purpose of this experiment, more information was obtained on soilage. Past research on soilage at the California station (Ittner *et al.*, 1954; Meyer *et al.*, 1956; Hull *et al.*, 1957) can be criticized because soilage was not compared to pasture at the same stocking rate. This experiment, with its range of stocking rate, can compare soilage and pasture at the same stocking rate. The number of steers fed soilage per acre was 3.24. From figure 3 the production from pasture at 3.24 steers per acre was estimated. These data are shown in table 3. In all cases, a greater quantity of digestible energy or forage was consumed per acre and more beef or corrected carcass was produced per acre from soilage. The corrected carcass production was 32% greater for the soilage than for the pasture treatment. Thirty percent has been about the average increase found by the California station in other experiments (Meyer *et al.*, 1958). This, plus a smaller decline in forage production in the latter part of the season from the fields used for soilage (figure 1), shows that soilage should realize, without a doubt, the greatest production per acre from forage.

TABLE 3. COMPARISON OF SOILAGE AND PASTURE

Item	Pasture	Soilage
Animals grazed per acre	3.24	3.24
Consumption per acre:		
Forage, lb.	5700	7351
Digestible energy, mcal.	6950	9262
Production per acre:		
Beef, lb.	575	609
Corrected carcass, lb.	425	561

Of interest here was the relative comparisons of the various measures used. Forage and digestible energy consumption were increased 29% and 33%, respectively, by soilage. Beef production per acre, however, showed only a 6% increase for soilage even though corrected carcass production increased 32%. These data might be interpreted to mean that feed intake and a measure equivalent in energy value, the corrected carcass, are more comparable reliable measures in experiments of this type.

Summary

A study was made of grazing intensity effects by imposing on irrigated pasture stocking rates of 1.35, 2.25, 3.06, 3.93 and 4.68 steers per acre. A group of steers fed soilage from the same pastures served as a positive control similar to what might be expected from pasturing forage without the superimposed animal influence. To increase the precision of the comparisons, body composition, intake and digestibility of the forage were added to the usual measurements of weight gains, clipping yields and chemical composition of the forage.

Increased stocking rate decreased yield of the forage as the season progressed. No carryover influence, however, was found the following season. Yield of forage from the soilage treatment was greater during the latter part of the season than from pasture treatments. The digestible energy content of the forage consumed by the soilage steers was 126 mcal. per 100 lb. For pasturing, a light stocking rate allowed the steers freedom to select a more nutritious forage, 130 to 132 mcal., whereas heavy stocking forced the steers to consume a less nutritious forage, 122 mcal. per 100 lb.

As grazing intensity increased from 1.35 to 4.68 steers per acre, feed and energy intake decreased, weight gain decreased and the energy content of the carcass decreased. In this pasture experiment maximum production per animal was not the proper measure. Production per acre was more realistic. As stocking rate increased the animals harvested and consumed more of the forage and digestible energy per acre even though consumption decreased per animal. A most important animal-plant interaction was present because liveweight gain and corrected carcass production per acre increased to a point, about four animals per acre, and then rapidly decreased. Increasing the stocking rate increased consumption of forage per acre, but here also consumption per animal was low enough so that too much forage was used for maintenance rather than gain and per-acre production decreased as a consequence.

A wide range in grazing intensity, 2.25 to

4 steers per acre, allowed close to maximum corrected carcass production per acre. This seems to indicate that a pasture operator has a wide latitude in stocking rate. A highly significant correlation (0.85) was found between digestible energy intake and forage available per animal as determined by pregrazing clipping.

Soilage compared to pasturing at the same stocking rate resulted in greater per-acre consumption of digestible energy and a 32% increase in corrected carcass production.

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SELECTION INDEXES FOR WEANLING TRAITS IN BEEF CALVES [1, 2]

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A PROBLEM arises when an animal breeder desires to select for more than one trait at the same time and the economic value is a function of the several traits. Hazel and Lush (1942) showed theoretically that selection on the basis of a total score or index should be a more efficient method of using the additive genetic variance than selection based on one trait at a time or selection based on several traits using independent culling levels. Smith (1936) and Hazel (1943) provided a quantitative expression in which each of the traits affecting net merit receives proper weight.

The purpose of this study was: (1) to develop a selection index based on growth and type score for weanling beef calves applicable in Virginia that should result in maximum genetic and economic progress per generation; (2) to compare the index which should bring about maximum improvement in both traits with (a) an index based on growth alone, and (b) an index based on type score alone; and, (3) to compare the three indexes mentioned with an index equivalent to the one being used by the Virginia Beef Cattle Improvement Association.

Stated in a practical way, this study would shed some additional light on the question of the relative emphasis to assign growth and body type in selecting beef calves at weaning. Such indexes were needed to evaluate current research being carried out at the Beef Cattle Research Station, Front Royal, Virginia, and to advise those interested in applying selection indexes in the field.

Selection indexes for beef cattle have been reported by few workers. Hazel (1952, unpublished) constructed an index using assumed values for the necessary parameters. His purpose was to show how an index could be constructed when accurate estimates of the necessary parameters became available. Lindholm and Stonaker (1957) constructed an index based on 118 Hereford steers, using weaning weight, gain from birth to weaning, weaning grade, postweaning gain, postweaning feed efficiency, and slaughter grade as the criteria for selection. Their purpose was to develop an index applicable in combined calf raising and finishing enterprises. Kincaid (1955, unpublished) constructed a total score index for weanling beef calves based on standard deviations estimated from approximately 300 calves and on assumed values of genetic and phenotypic relationships. This index was designed for use in a beef cattle performance testing program, and was arbitrarily computed such that it would give equal emphasis to phenotypic standard deviations of average daily gain and type score. It is presently being used by the Virginia Beef Cattle Improvement Association and approximately 30 similar organizations. As far as the authors of the present study could determine, this study is the first in which Hazel's (1943) multiple correlation method of constructing selection indexes has been applied to a large body of beef cattle data.

Materials and Methods

Growth and Body Type Data. The data used in this study were collected from the Angus, Hereford, and Shorthorn herds at the Beef Cattle Research Station, Front Royal, Virginia; the station is operated cooperatively by the Virginia Agricultural Experiment Station and the Agricultural Research Service of the United States Department of Agriculture. Information suitable for use in this study was available on the calf crops born from 1950 through 1958, comprising a total of 1,987 calves. There were 606 Angus, 656 Hereford, and 689 Shorthorn calves, all purebred, and 36 grade Hereford calves; they were the offspring of 33 Angus, 35 Hereford, and 37 Shorthorn bulls. Little selection was practiced

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[2] Contribution to Southern Regional Beef Cattle Breeding Project S-10.

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for production traits during the years covered by this study. There were some deviations from random mating, but these were thought to be relatively unimportant with respect to this study. This sample was considered typical of one from actual farm conditions in Virginia.

All calves were scored for body type (by a committee of three or more men) to the nearest one-third of a federal-state grade designation, using a value of ten for middle good, and adding or subtracting one for each one-third of a grade increase or decrease, respectively.

Economic Values. The data used to arrive at an estimate of the value of a unit increase in growth rate were collected from reports of the Cooperative Virginia Feeder Calf Sales over a 16-year period (1943 through 1958) and involved nearly a quarter of a million calves. Yearly averages were compiled for each sex, and an unweighted average over years and sexes was computed. This method yielded a figure of 22 cents for the value of one pound of weanling calf.

Daily gain has no economic value as such; therefore, it was necessary to transform the value per pound such that it would furnish an economic value of daily gain. This was done according to the following procedure: (1) the value of a weanling calf was found to be $89.96, arrived at by multiplying the value per pound (22 cents) by the average weaning weight (402 lb.) of all calves used; (2) the mean daily growth rate of all calves was 1.65 lb. per day, computed by subtracting the average birth weight (67 lb.) from the average weaning weight (402 lb.) and dividing by the average age at weaning (203 days); (3) the mean daily growth rate was divided into the value per weanling calf, and resulted in an estimate of $54.53 as the value of a pound of daily gain (rounded to $54.50). This procedure permitted the use of daily gain as a selection criterion.

The economic value of a unit change in federal-state grade was compiled from the Feeder Calf Sales reports for the years 1951 through 1958, pertaining to approximately 185,000 calves. There was a small number of fancy calves, so these were pooled with choice calves; the sales during 1951–58 did not sell calves below the grade of medium. Therefore, a price differential could be obtained between choice and good, and between good and medium. This restriction in range of grades was not considered serious, because the great majority of Front Royal calves used in this study fell within the medium, good, and choice grades. The value was calculated as follows: (1) the average values per hundred weight for the grades choice, good, and medium were computed; (2) differences between grades (choice minus good, and good minus medium) were weighted by the proportion of calves in the respective grades to obtain the average increase in value, per hundred weight, for one grade improvement ($2.33); (3) this value was multiplied by 402, the herd average weaning weight, and divided by three, in order to obtain a value of $3.12 for one grade point (one-third of a federal-state grade) improvement in the weanling calf (rounded to $3.00 in the indexes). This meant using feeder grade prices to estimate a value of type score change, but this was not considered detrimental to the study. The value estimated as described was felt to be quite realistic.

Methods. Year, sex, breed, age of dam, and month of birth were considered fixed environmental effects. Since the data were non-orthogonal, constants for these effects were obtained by conventional least squares methods. Study of the unadjusted means indicated there were no practically significant interactions between the effects named, so they were assumed to be zero. A punched card was prepared for each calf, and the data were adjusted for all effects mentioned above.

Heritabilities and their standard errors were obtained from paternal half-sib comparisons according to the method of Hazel and Terrill (1945). This method assumes that the dams are unrelated and that the sires are a random sample from an unselected population. The data for this study were taken from the early years of the breeding project at Front Royal, before much relationship between the females was built up, and before it was possible to practice much selection for sires; therefore, it was assumed that the method mentioned would yield valid estimates of heritability.

Phenotypic correlations were calculated by applying the standard product-moment method to the adjusted data.

Estimates of genetic correlations were obtained from paternal half-sib analyses using the sire components of variance and co-variance, as developed by Hazel (1943). These correlations were obtained from data that were adjusted for the average effects of year, sex, breed, age of dam, and month of birth, so it was hoped that the components for these effects were not included in the sire components.

Selection indexes for different combinations

of the traits studied were calculated using the parameters estimated in this study and the multiple correlation method (Hazel, 1943). The partial regression coefficients (b values), the correlations between the indexes and the aggregate genetic values (R_{IH}), and the expected genetic changes per standard deviation change in index were also computed.

Results and Discussion

Table 1 contains estimates of the constants for fixed effects from least squares analysis.

TABLE 1. LEAST SQUARES ESTIMATES OF CONSTANTS AND STANDARD ERRORS

Constants for	No. calves	Weaning wt.	Daily gain	Type score
Breed:				
Angus	606	40±4	.23±.01	.7±.0
Hereford	656	7±1	.05±.01	.1±.0
Grade Hereford	36	10±3	.11±.05	−.5±.1
Shorthorn[a]	689	0	.00	.0
Sex:				
Heifers[a]	992	0	.00	.0
Bulls	802	41±2	.18±.01	−.2±.0
Steers	193	6±1	.01±.02	−.9±.1
Age of cow:				
2	52	−32±3	−.13±.01	−.7±.1
3[a]	383	0	.00	.0
4	352	20±2	.10±.02	.2±.1
5	299	33±2	.14±.02	.5±.1
6	250	46±2	.20±.02	.6±.1
7	176	32±1	.13±.02	.3±.1
8	151	33±2	.14±.02	.4±.1
9	122	22±2	.10±.03	.2±.1
10	65	22±3	.10±.03	−.3±.1
11	53	11±3	.06±.03	−.2±.1
12	31	−8±5	−.03±.05	−.5±.1
13	23	26±5	.10±.05	−.6±.2
14	30	4±3	−.03±.05	−.4±.1
Month born:				
January	322	−16±2	−.09±.02	.0
February[a]	870	0	.00	.0
March	482	8±3	.03±.01	.0
April	222	16±3	.06±.02	−.4±.1
May	65	13±4	.04±.03	−.4±.1
June	26	−3±3	−.04±.05	−.9±.2

Constants for years were computed, but are not shown in table.

[a] Base equations—the constants are deviations of each group from the mean of the base group for each classification.

These effects had a greater influence on daily gain and weaning weight than on type score.

Heritabilities of the various traits, estimated from paternal half-sib correlations, were computed for three traits, average daily gain, weaning weight, and body type. The estimate for daily gain (0.20±.03) is within the range of zero and 0.45 published by Gregory *et al.* (1950), and is essentially the same as the estimate of 0.21 found by Koch and Clark (1955 b). Weaning weight (203-day weight in this study) was estimated to be 0.21±.03 heritable. This estimate is in close agreement with values of 0.24 and 0.19 shown by Koch and Clark (1955 b, 1955 d). The value of 0.33±.04 for type score is between the estimate of 0.53 found by Knapp and Nordskog (1946 b) and estimates of 0.18 and 0.16 reported by Koch and Clark (1955 b, 1955 d). Rollins and Wagnon (1956 b) reported an estimate of 0.36, very close to the one found in this study.

TABLE 2. ESTIMATES OF CORRELATION COEFFICIENTS

Item	Weaning weight	Type score
Daily gain		
Genetic	0.93	0.007
Phenotypic	0.97	0.42
Weaning weight		
Genetic	....	−.026
Phenotypic	....	0.39

The genetic and phenotypic correlations found in this study appear in table 2. The estimates of genetic correlation between daily gain and type (0.007) and between weaning weight and type (−.026), indicated that growth and type are essentially genetically independent. On the other hand, the phenotypic correlations (0.42 and 0.39) lead to the conclusion that weight (probably degree of finish) and body type were positively associated in the eyes of the graders.

The economic values mentioned above and the estimates of genetic and phenotypic variances and covariances shown in table 3 were used to compute the eight selection indexes presented in table 4. The first four considered daily gain the measure of growth, and the aggregate genetic value to which they were correlated was $H_1=54.50\ G_1+3.00\ G_3$ ($\hat{\sigma}H=5.81$) where G_1 and G_3 were the expected measures of the traits due to additive gene action. Considering the first four indexes, index 1 was the best linear estimate of an animal's net worth, giving a phenotypic proportion of approximately 32 to 1. Index 2 was based on daily gain alone; index 3 was based on type

TABLE 3. ESTIMATES OF VARIANCE AND COVARIANCE

Item	Daily gain	Weaning weight	Type score
Daily gain			
Genetic	0.0109	2.2628	0.0006
Phenotypic	0.0562	11.7048	0.1455
Weaning weight			
Genetic	.....	547.5492	−.5372
Phenotypic	.....	2597.5931	29.0353
Type score			
Genetic	.....	.....	0.7236
Phenotypic	.....	.....	2.1818

TABLE 4. SELECTION INDEXES USING VARIOUS COMBINATIONS OF THE TRAITS STUDIED

Index no.	b values for index			R_{IH}	Expected genetic changes		
	Daily gain	Weaning weight	Type score		Daily gain	Weaning weight	Type score
1	10.79	...	0.34	0.41	0.043	.....	0.09
2	1.00	...	0.00	.24	.047	.....	.003
3	0.00	...	1.00	.24	.000	.....	.49
4	6.25	...	1.00	.25	.028	.....	.29
5	....	1.0	8.71	.43	....	9.50	.10
6	....	1.0	0.00	.42	....	10.74	.00
7	....	0.0	1.00	.25	....	−0.36	.49
8	....	1.0	34.00	.40	....	6.28	.29

alone; and index 4 gave equal emphasis to standard deviations of the two traits. Comparisons among these four indexes on the basis of their correlations with the aggregate genetic values and the expected changes in genetic value per standard deviation change in index indicated that index 1 was definitely preferable to 2, 3 and 4. Index 4 was very little better than 2 or 3 in R_{IH}, which was due to the very low estimate of genetic covariance betwen the two traits (table 3). If this covariance estimate is anywhere near the true value, then the index being used in some performance testing programs is very little better (in terms of genetic progress for maximum economic return) than would be an index based on either trait singly. However, it must be considered that index number 4 should result in some genetic change in both traits, whereas No. 2 would allow effectively no progress in type and No. 3 would allow effectively no progress in growth rate.

Indexes 5, 6, 7 and 8 were based on weaning weight and type score; the aggregate genetic value with which these indexes were compared was $H_2=0.22\ G_2+3.00\ G_3$ ($\hat{\sigma}H=5.56$) where G_2 and G_3 were the expected measures of the traits due to additive gene action. The estimated genetic correlation between daily gain and 203-day weight was 0.93, indicating that the genes for the two measures of growth were quite closely associated; then it became of interest to see whether indexes expressed in terms of 203-day weight and type would lead to the same conclusions as those expressed in terms of daily gain and type. When the results were completed, the authors felt the expected genetic changes for indexes 5, 6, 7 and 8 had the same meaning as those for indexes 1, 2, 3 and 4 (the one negative value is small, and could be considered zero). The R_{IH} values did not show very close agreement between numbers 2 and 6, and numbers 4 and 8, indicating two things: (1) selection on the basis of weaning weight alone should give better results than selection on the basis of daily gain alone, and (2) an equal emphasis index using weaning weight as the measure of growth should result in more genetic and economic improvement per unit of time than an equal emphasis index using daily gain as the measure of growth. Daily gain is a measure of the rate of gain from birth to weaning; 203-day weight is a measure including the number of pounds gained from conception to birth and from birth to weaning. It is conceivable that this natural difference could cause some differences in the correlations mentioned, but it did not seem likely to the authors that this natural difference could account for discrepancies as large as those shown (especially with the genetic correlation being 0.93). Therefore, the authors concluded that the R_{IH} differences between indexes 4 and 8 were due more to the large genetic variance of 203-day weight and the arbitrary nature of the particular indexes, and less to the biological differences in the measures of growth. This conclusion was strengthened by the fact that the two properly weighted indexes (1 and 5) were quite similar as predictors of results from selection.

The relative emphasis given growth rate and type in terms of expected economic gain per phenotypic standard deviation of selection deserved some consideration, in order to show that the relative phenotypic emphasis given the traits in an index does not necessarily express the extent to which one trait is favored and the other discriminated against.

Consider, for example, that the relative phenotypic emphasis expressed the fact that index four discriminated against type in the ratio of 6.25 to 1. The standard deviation of daily gain was found to be 0.24, and the value was $54.53 per lb., so the economic value of

daily gain per standard deviation was $13.08. Using the heritability of 0.20, the expected economic increase per standard deviation of phenotypic selection was $2.62 (for 203-day weight the increase was $2.36). A similar procedure with type using a standard deviation of 1.5, economic value of $3.00 per grade point, and heritability of 0.33, yielded a value of $1.48. The relative value ($2.62 to $1.48) was then $1.77 to $1.00. Therefore, it should be understood that while index 4 gave a relative phenotypic emphasis of 6.25 to 1, the relative emphasis in terms of economic gains to be expected from selection based on index 4 was reduced to approximately 2 to 1. In terms of phenotypic standard deviations, index 1 gave approximately five times as much emphasis to daily gain as it did to type. Five standard deviations of daily gain would be worth $65.40, and when multiplied by a heritability of 0.20, should yield an economic return of $13.08. Compared with $1.47 increase from selection for type, index 1 gave a relative figure of approximately 9 to 1. Therefore, whereas index 1 gave a phenotypic ratio of 32 to 1, the ratio of the two traits in terms of expected economic increases resulting from selection was reduced to 9 to 1.

In areas where the beef cattle population includes contrasting kinds with respect to genetic background and conformation (i.e., British, Brahman, British-Brahman crosses and top crosses of British and Brahman on native stocks) the standard deviation of type score would be expected to be larger than was observed in this study. If such a population had a standard deviation for type score amounting to one full grade and if the price differential between grades tended to increase as the grade moved downward from good, the relative emphasis in terms of expected economic increase might be in favor of type. This would, of course, first change the relative phenotypic emphasis in the selection indexes.

This study was based on the largest sample of data that was available at the Virginia station. It can be questioned because: (1) the economic values of bulls and heifers at weaning are usually different (especially in purebred herds), (2) the parameters computed may be different in the different breeds and sexes, and (3) the indexes and discussion pertaining to performance testing should be compared with indexes computed using field data. This is the first study of this kind based on growth rate and type of weanling beef calves. Therefore, application of the results of this study should await results of studies based on single breeds and sexes.

Summary

Eight selection indexes for weanling beef calves were developed and compared. These were based on daily gain from birth to weaning, 203-day weight, and type score.

Selection on the basis of an index combining daily gain and type or weaning weight and type in the best linear equation was concluded to be preferable to selecting on the basis of either growth or type singly.

Estimates of heritabilities, genetic and phenotypic correlations, and economic values were calculated.

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FACTORS AFFECTING THE DIGESTIBILITY OF FLAX PLANT PRODUCT BY SHEEP

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FLAX plant product is that portion of the flax plant remaining after harvesting the seed and separation of the bast fibers and flax shives. It consists of the leaves, cortical tissues, flax seed bolls and broken and immature flax seeds. This feedstuff was known previously as flax plant feed and flax plant by-product. It was described and its present name approved by the Association of American Feed Control Officials (1960) in 1957.

A review of the literature revealed a lack of information on the digestibility of flax plant product by ruminants. Preliminary studies of digestibility and feeding value were made by Hanson *et al.* (1953). They found that yearling sheep would consume sufficient quantities of flax plant product to meet their dry matter requirements (Morrison, 1956) to maintain weight when the feed was pelleted with 10% added moisture. The average apparent digestion coefficients ranged from 29% for fiber to 43% for protein, and the TDN value was about 30%. Since this feedstuff is fed only in mixed rations, Kolari (1957), using the miniature artificial rumen, studied the effect of various feed ingredients on the digestibility of fiber in flax plant product.

The study reported herein was based on the laboratory findings with the artificial rumen and was designed to measure the digestibility by sheep of flax plant product when fed as the principal constituent in various combinations with other feedstuffs.

Experimental Procedure

Three lots of three wether sheep were used in digestion trials during four experimental periods in which flax plant product was the principal feedstuff in 11 test rations. Timothy hay was fed to one lot of three sheep as a negative control. All rations were pelleted. The sheep were lotted initially on a weight basis, average weight being 78 lb.; thereafter, they remained in their respective lots during four trial periods.

The wethers were weighed at the start of the two-week preliminary period preceding each trial. They were weighed again at the beginning and end of 14-day collection periods. The wethers were individually fed twice daily. The feed allowance of each sheep was adjusted to the maximum that would be consumed. Steamed bone meal was added to all rations to meet National Research Council recommendations (1949) for calcium and phosphorus. Vitamins A and D were added at levels of 600 and 60 I.U. per pound of feed, respectively. Water and salt were available *ad libitum* except when the wethers were being fed. Clean, dry plasterers sand was used as bedding.

A bag and harness described by Cook *et al.* (1952) was used to collect feces; it was patterned after equipment supplied by L. E. Harris, Utah State University. Fecal bags were emptied twice daily and a uniform 1.0% sample representative of each sheep's daily output of feces was stored in a frozen state in polyethylene freezer bags. Representative samples of the test rations were obtained every other day and stored in a similar manner. Chemical analyses were made on uniform samples of pooled feed and feces upon completion of the trials.

Fecal samples collected from the sheep prior to the start of the trials and analyzed in the parasitology laboratory, College of Veterinary Medicine, University of Minnesota, revealed no harmful parasites.

Moisture determinations were made by the method of Swanson and Sloan (1953) and the remaining proximate analyses were conducted according to A.O.A.C. methods (1950). The data were analyzed statistically by the analysis of variance. Duncan's multiple range test (1955) was used to determine significance of differences between means.

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TABLE 1. COMPOSITION OF RATIONS

Trial number	I			II			III			IV		
Ration number[a]	1	2	3	4	5	6	7	8	9	10	11	12
Ingredient, %												
Flax plant product	100.0	85.0	70.6	80.0	65.0	50.0	78.25	55.0	50.5	....	55.0	51.0
Corn, shelled	....	....	13.1	20.0	20.0	32.0	20.0	20.0	20.0	....	20.0	20.0
Soybean oil meal	....	15.0	1.5	....	15.0	1.5	....	15.0	10.0	....	10.0	10.0
Linseed oil meal	....	....	1.0	....	....	1.0	....	....	5.0	....	5.0	5.0
Molasses	....	....	8.3	....	....	10.0	....	10.0	10.0	....	10.0	10.0
Urea	....	....	1.5	....	....	1.5	1.75	....	0.5	....	....	....
Alfalfa meal	....	....	4.0	....	....	4.0	....	....	4.0	....	....	4.0
Timothy hay	....	....	....	....	....	....	....	....	....	100.0	....	....
Chemical composition, %												
Dry matter	89.3	89.4	89.9	89.4	89.4	88.2	89.5	88.5	87.2	93.1	88.9	88.0
Organic matter	84.7	83.4	85.0	85.5	87.4	87.9	87.5	86.6	87.9	92.1	89.1	89.2
Crude fiber	27.0	26.6	24.5	28.6	23.3	17.8	24.3	19.8	18.6	26.2	19.6	19.2
Crude protein	13.2	16.6	14.9	10.6	16.0	13.8	14.7	16.1	16.6	9.0	16.0	15.1
Ether extract	3.2	2.4	3.0	2.9	2.6	3.2	3.0	2.9	2.8	2.4	2.7	2.9
Ash	15.3	16.7	14.9	14.5	12.7	12.1	12.5	13.4	12.1	7.9	10.9	10.8
N-free extract	41.3	37.8	42.6	43.4	45.5	53.1	45.5	47.8	49.9	54.5	50.8	52.0

[a] Calcium Carbonate Co. trace mineral premix added at 0.05% to supply the following elements in parts per million of experimental rations: manganese from manganese sulfate, 60; iron from ferrous sulfate, 27; iron from ferrous oxide, 21; copper from copper carbonate, 3.7; cobalt from cobalt sulfate, 1.3; iodine from potassium iodide, 1.9; zinc from zinc sulfate, 4.5. Steamed bone meal added to provide a minimum of 4.5 grams phosphorus and 10 grams calcium per sheep daily. Vitamin A and D added to supply 600 and 60 I. U. per pound of feed, respectively.

Results and Discussion

The physical and chemical composition of rations used is given in table 1. The apparent digestibility and TDN value of the 12 experimental rations and significance of differences between means of digestion coefficients for the various nutrients are shown in table 2.

The apparent digestion coefficients of flax plant product supplemented with minerals and vitamins (ration 1) were: dry matter, 33.6; fiber, 13.8; protein, 53.8; ether extract, 48.4 and nitrogen-free extract, 49.1. The low apparent digestibility, particularly of fiber, may be partially explained by the high lignin content (17.8%) [3] of this sample of flax plant product. Lignification is associated with advancing maturity of forages.

Replacement of 20% flax plant product by shelled corn (ration 4) significantly ($P<0.05$) increased the digestibility of ration crude fiber from 13.8% to 23.1%. The digestibilities of dry matter and nitrogen-free extract were increased only slightly. Likewise, the replace-

[3] Analyses by M. O. Schultze, Department of Agricultural Biochemistry.

TABLE 2. NUTRIENT DIGESTIBILITY AND TDN CONTENT OF FLAX PLANT PRODUCT AND OF RATIONS CONTAINING THIS FEEDSTUFF AS DETERMINED WITH SHEEP

Trial number	Ration number	Dry matter	Organic matter	Crude fiber	Crude protein	Ether extract	N-free extract	TDN
		%	%	%	%	%	%	%
	1[a]	33.6[a]	38.6[ab]	13.8[abc]	53.8[ab]	48.4[fg]	49.1[ab]	34.6
	2	32.9[a]	37.4[ab]	16.7[abc]	55.8[ab]	25.2[a]	44.6[a]	31.9
	3	30.9[a]	35.8[a]	8.4[a]	48.4[a]	36.1[cde]	47.2[ab]	31.8
2	4	37.4[ab]	41.3[ab]	23.1[cd]	39.0	42.8[efg]	53.3[bc]	36.6
	5	45.1[c]	49.5[cd]	19.9[bc]	58.8[b]	40.6[def]	61.8[de]	44.5
	6	55.9[e]	61.0[f]	30.9[d]	57.0[b]	48.8[g]	72.6[g]	55.4
3	7	36.9[ab]	42.9[bc]	10.7[ab]	55.2[ab]	29.2[abc]	57.7[cd]	40.0
	8	44.4[c]	50.6[d]	16.6[abc]	55.0[ab]	28.4[ab]	64.6[ef]	44.8
	9	43.3[bc]	48.8[cd]	9.9[ab]	55.9[ab]	34.8[bcd]	62.1[de]	44.3
4	10[a]	46.7[cd]	48.6[cd]	23.4[cd]	53.2[ab]	44.2[fg]	60.1[de]	46.0
	11	48.3[cd]	52.7[de]	15.9[abc]	60.2[b]	42.5[defg]	65.6[ef]	49.7
	12	52.5[de]	57.5[ef]	22.3[cd]	58.5[b]	45.6[fg]	70.8[fg]	52.9

[a] Flax plant product was the only feedstuff in Ration no. 1 and timothy hay was the only feedstuff in Ration no. 10. Both rations were supplemented with vitamins A and D and minerals as indicated in footnote a, table 1.

a, b, c, d, e, f, g Any two means with the same letters within columns are not significantly different at the 1% level of probability.

ment of 32% flax plant product by corn (ration 6) significantly ($P<0.01$) increased the digestibility of all ration nutrients when compared to ration 3. These two rations were similar except that ration 3 contained 13% corn, 71% flax plant product as compared to 50%, and slightly less molasses. Results of the sheep digestion trials of Phillips *et al.* (1951) indicated an increased digestibility of all ration nutrients due to the supplementation of alfalfa with corn and are generally similar to the results reported herein. Lassiter *et al.* (1955) reported only a slight increase in the digestibility of dry matter and a significant increase in the digestibility of crude protein of a low quality timothy hay ration by dairy steers as a result of corn supplementation. However, they reported that corn significantly decreased the digestibility of crude fiber.

The replacement of 10% flax plant product with molasses in a ration containing 20% corn and 15% soybean oil meal (ration 8) had little effect upon digestibility. However, compared with ration 5 the digestibility of ether extract was decreased significantly ($P<0.01$). These results are in accord with those of Embry *et al.* (1955) who fed early and late-cut prairie hay supplemented with a concentrate mixture containing 0%, 10% or 20% molasses. They found little, if any, effect of molasses additions upon ration nutrient digestibility.

Several authors have reported a depression in cellulose and fiber digestibility due to molasses in ruminant rations (Bell, *et al.*, 1953a, 1953b; Hoflund *et al.*, 1948; Johnson, *et al.*, 1942). On the other hand, Clark and Quin (1951) reported an increase in the digestibility of cellulose when 6% and 10% molasses were included in a ration of poor quality hay and 2% urea fed to sheep.

The supplementation of flax plant product with 15% soybean oil meal increased digestibility of crude fiber from 13.8% to 16.7% (ration 2 vs. 1), and decreased the digestibility of ether extract ($P<0.01$) from 48.4% to 25.2%. Differences between the two rations in digestibility of other ration nutrients were not significant. The relatively high percentage of protein in the flax plant product (13.2%) may explain the lack of response to supplementation with 15% soybean oil meal. The results of Briggs and Heller (1942), in which the addition of cottonseed meal to an alfalfa hay ration did not improve fiber digestibility, lend support to the above suggestion.

The inclusion of 15% soybean oil meal (ration 5) in a mixture of 65% flax plant product and 20% corn (ration 4) increased ($P<0.01$) the apparent digestibility of dry matter, organic matter, protein and nitrogen-free extract.

Five percent linseed oil meal in ration 11 replaced an equivalent amount of soybean oil meal in ration 8. This change in source of supplemental protein was without effect upon the apparent digestibility of all ration nutrients except ether extract.

The supplementation of a flax plant product-corn mixture with 1.75% urea decreased ($P<0.01$) the digestibility of crude fiber and ether extract (ration 7 compared to ration 4); it increased ($P<0.01$) the apparent digestibility of protein from 39.0% to 55.2%. Bell *et al.* (1953b) observed a significant increase in the digestibility of protein but no effect on other nutrients, as a result of the supplementation of 7%-8% protein basal rations with urea. However, Swift *et al.* (1947) found that the addition of urea to a 12.9% protein ration decreased digestibility of crude fiber and ether extract, but increased apparent digestibility of protein.

A comparison of the digestion coefficients of the flax plant product-corn-soybean oil meal and the flax plant product-corn-urea rations (5 vs. 7) indicates that soybean oil meal supplementation resulted in consistently higher nutrient digestibility. The results of Bell *et al.* (1953, 1955) are in accord with these findings.

Ration 11 contained flax plant product, corn, soybean oil meal, linseed oil meal and molasses. Ration 12 was similar to ration 11 except that alfalfa meal replaced 4% of flax plant product. Nutrient digestibility was slightly increased by the small amount of alfalfa meal in ration 12. Results of a number of studies indicate that alfalfa exerts a favorable effect upon nutrient digestibility by ruminants (Burroughs *et al.* 1950; Bentley *et al.*, 1952; Chappel *et al.*, 1955). However, results by Embry *et al.*, (1955) are not in agreement.

The digestibilities of dry matter, organic matter and nitrogen-free extract were higher ($P<0.01$) in timothy hay (ration 10) than in the flax plant product (ration 1); so was the digestibility of fiber ($P<0.05$).

No difficulty was experienced in starting or maintaining the wethers on pelleted flax plant product, or mixtures containing this feedstuff, indicating satisfactory palatability. The average daily gain of all sheep for all treatments during these trials was 0.27 lb. which was con-

sidered satisfactory under the conditions of the experiment.

Summary

Three lots of three sheep each were used in digestion trials in four experimental periods in which flax plant product was fed as the principal feedstuff in 11 test rations. Timothy hay was fed in one ration as a negative control. The effect of the addition of corn, molasses, soybean oil meal, linseed oil meal, urea, and alfalfa meal, alone or in various combinations, upon the digestibility of nutrients in flax plant product rations was tested.

The apparent digestibility of flax plant product supplemented with vitamins and minerals, when fed as the sole feedstuff to sheep, was low. Its total digestible nutrient content was 34.6%. The inclusion of corn in flax plant product rations exerted a favorable effect upon nutrient digestibility.

Addition of soybean oil meal to flax plant product had little effect upon ration nutrient digestibility. However, the addition of soybean oil meal plus corn significantly ($P<0.01$) increased the digestibility of dry matter, organic matter and nitrogen-free extract. Molasses added to a ration containing flax plant product, corn and soybean oil meal apparently provided no additional factor(s) stimulatory to nutrient digestibility. The addition of 1.75% urea to a flax plant product-corn ration resulted in a significant ($P<0.01$) decrease in fiber digestibility and a significant ($P<0.01$) increase in protein digestibility.

Alfalfa meal additions to rations composed principally of flax plant product increased the digestibility of most nutrients.

The average daily gain (0.27 lb. per day) of the test wethers fed pelleted flax plant product rations throughout the four trial periods was satisfactory.

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THE GROWTH-PROMOTING FACTORS IN DRIED CORN DISTILLERS SOLUBLES AND POSSIBLE INTER-RELATIONSHIPS BETWEEN SOLUBLES AND CERTAIN MINERALS [1]

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NUMEROUS workers have demonstrated the adverse effects of feeding high levels of calcium to swine (Kernkamp *et al.*, 1953; Lewis *et al.*, 1955; Conrad *et al.*, 1957). Tucker and Salmon, (1955) Luecke *et al.* (1956) and Lewis *et al.* (1956) have demonstrated that parakeratosis in swine can be prevented or alleviated by the addition of zinc to the ration. Conrad *et al.* (1957) reported that distillers solubles gave complete protection against parakeratosis. Other workers have reported the supplementary value of distillers dried solubles (Barnhart, 1952; Catron, 1954).

The purpose of this experiment was: (1) to study the effect of various levels of minerals and (2) to determine the growth promoting factors in dried corn distillers solubles and their possible interrelationship with zinc, specifically, and possibly other trace minerals.

The first experiment was conducted during the summer of 1957, the second during the fall of 1957 and the third during the winter of 1957–58. Statistical tests were made by the analysis of variance.

Experimental Procedure

Experiment 1. Thirty-two Hampshire weanling pigs averaging 26 lb. were allotted by balancing from outcome groups of four pigs to eight ration treatments. Four pigs were individually fed each ration and were confined to individual wire-floored metal crates which had previously been painted with a non-metalic paint. Each crate was equipped with a self-feeder and automatic waterer. The experimental treatments were as follows: (1) basal ration (table 1), (2) basal plus 5% dried corn distillers solubles, (3) basal plus 0.57% calcium (supplied as ground limestone), (4) treatment 3 plus 5% distillers solubles, (5) basal plus 26.7 ppm of zinc (supplied as zinc sulfate), (6) treatment 2 plus 26.7 ppm of zinc, (7) treatment 3 plus 26.7 ppm of zinc, (8) treatment 4 plus 26.7 ppm of zinc. The addition of distillers solubles or calcium replaced an equal portion of starch. All pigs were fed their respective rations for 53 days.

Experiment 2. Since no symptoms of parakeratosis were observed in experiment 1, a higher level of calcium was fed in experiment 2.

Thirty-six early-weaned Hampshire pigs averaging approximately 13 lb. were allotted from outcome groups of four pigs to nine ration treatments. Four pigs were individually fed each ration and were confined to individual crates as in experiment 1. This experiment ran for 8 weeks. The room temperature was maintained between 58° and 64° F.

The experimental treatments were as follows: (1) basal ration, (table 1), (2) basal plus 5% distillers solubles, (3) basal plus 0.83% calcium, (4) treatment 3 plus 5% distillers solubles, (5) basal plus 26.7 ppm of zinc, (6) treatment 2 plus 26.7 ppm of zinc, (7) treatment 3 plus 26.7 ppm of zinc, (8) treatment 4 plus 26.7 ppm of zinc, (9) treatment 3 less 2% corn oil (Mazola). The addition of distillers solubles and calcium was made as in experiment 1.

Experiment 3. Sixty-four Hampshire barrows and gilts averaging about 38 lb. were allotted by balancing from outcome groups of four pigs to eight ration treatments with two replicate lots of four pigs.

The pigs were kept in concrete-floored pens and had access to outside runways. A self-feeder and automatic waterer were used in each pen.

The experimental treatments were as follows: (1) basal ration (table 1), (2) basal plus 5% distillers solubles, (3) basal plus 0.89% calcium, (4) treatment 3 plus 5% dis-

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director.

[2] This material is based upon part of a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree at the University of Kentucky.

TABLE 1. COMPOSITION OF BASAL RATIONS FED IN EXPERIMENTS 1 AND 2 [a] AND IN EXPERIMENT 3 [a]

Experiments 1 and 2		Experiment 3	
Ingredients	Pounds	Ingredients	Pounds
Pearl starch	38.30	Ground yellow corn	77.60
Cerelose	30.00	44% soybean oil meal	20.50
Drackett protein	19.50	Ground limestone	1.00
Woodflock	2.00	Salt (mineral) [f]	0.50
Corn oil (Mazola)	4.00	Vitamin supplement [g]	.25
Trace mineral mix [b]	3.00	Vitamin A and D supplement [h]	.05
Di-Calcium phosphate	1.00	Vitamin B_{12} supplement [i]	.05
Vitamin premix [c]	2.00	Antibiotic premix [j]	.05
98% DL-Methionine	0.20		
Antibiotic [d]	+		

[a] Chemical analysis: protein 16.06%; fat 4.00%; fiber 2.00%; calcium 0.48%; phosphorus 0.38%; zinc, 40 ppm.
[b] Trace minerals added, grams per 100 lb. of ration: $MnSO_4$ (70% $MnSO_4$), 18.11; copperas ($FeSO_4 \cdot 7H_2O$), 81.72; $CuCO_3$ (55% Cu), 0.62; $CoSO_4$ (33% Co), 0.43; $ZnSO_4$ (36% Zn), 6.81; K_2SO_4, 401.79; KI-Ca Stearate, 0.027; NaCl, 231; $CaHPO_4$ (21% P), 392.12.
[c] Vitamins added per pound of ration: alpha-tocopherol acetate, 3.0 mg.; biotin, 0.2 mg.; ascorbic acid, 50.0 mg.; choline chloride, 450.0 mg.; calcium panthothenate, 5.0 mg.; folic acid, 0.5 mg.; niacin, 15.0 mg.; riboflavin, 3.0 mg.; thiamine HCl, 3.0 mg.; pyridoxine HCl 1.5 mg.; menadione, 1.0 mg.; p-aminobenzoic acid, 4.0 mg.; inositol, 200 mg.; 2-methyl-1-4-naphthoquinone, 1.05 mg.; vitamin B_{12} activity, 10.0 mcg.; vitamin A acetate, 3000 I.U.; and vitamin D_2, 400 I.U.
[d] Chlortetracycline HCl was added to supply 25.0 mg. per pound of ration.
[e] Chemical analysis: protein, 15.31%; fat, 3.75%; calcium, 0.47%; phosphorus, 0.43%; zinc, 40 ppm.
[f] The trace mineralized salt had the following composition, %: sodium chloride, 98.5 to 99.5; iodine, 0.007; iron, 0.09; manganese, 0.16; copper, 0.05; cobalt, 0.01; zinc, 0.005.
[g] One pound of the vitamin supplement supplied not less than 2 gm. riboflavin, 4 gm. panthothenic acid, 9 gm. niacin, 10 gm. choline chloride and not less than 60 mg. folic acid.
[h] One pound of the vitamin A and D supplement contained not less than 4,450,000 USP units of vitamin A and not less than 567,500 USP units of vitamin D_2.
[i] The vitamin B_{12} supplement contained 10 mg. vitamin B_{12} per pound.
[j] The antibiotic premix furnished 10 gm. of chlortetracycline hydrochloride per pound.

tillers solubles, (5) basal plus 26.7 ppm of zinc, (6) treatment 2 plus 26.7 ppm of zinc, (7) treatment 3 plus 26.7 ppm of zinc, (8) treatment 4 plus 26.7 ppm of zinc. Distillers solubles and calcium replaced a dietary portion of corn.

At the end of 37 days certain changes in rations were made to study the curative effect of zinc added to treatments 3 and 4 which had produced severe parakeratosis in 7 of the 16 animals; also, to determine if the animals which had been on a low-calcium diet (1 and 2) or a high-calcium diet (7 and 8) supplemented with zinc would develop parakeratosis.

Results

Experiment 1. The results are presented in table 2. The feeding of semi-purified rations containing two levels of calcium, (0.48% and 1.05%) two levels of zinc (40 ppm and 66.7 ppm) and with and without distillers solubles failed to result in any statistical significant differences in gain or feed conversion between the ration treatments.

Experiment 2. The results are summarized in table 2. Feeding pigs a semi-purified ration containing levels of calcium up to 1.31% failed to produce parakeratosis. Gains were decreased in pigs receiving the high-calcium (1.31%) diets not supplemented with zinc. The addition of 5% distillers solubles improved rate of gain slightly in all treatments except 4. The low-fat ration failed to increase the susceptability to parakeratosis of pigs on the high-calcium ration without supplemental zinc.

TABLE 2. RESPONSE OF PIGS TO TREATMENTS IN EXPERIMENTS 1 AND 2

Treatment	Av. initial wt., lb.	Av. daily gain, lb.	Feed per lb. gain, lb.
Experiment 1—53 days			
1 (Basal)	29.50	1.43	2.22
2 (1 plus CDS) [a]	26.50	1.48	2.13
3 (1 plus Ca)	26.20	1.36	2.41
4 (3 plus CDS)	25.50	1.44	2.39
5 (1 plus Zn)	25.50	1.32	2.34
6 (2 plus Zn)	25.50	1.44	2.23
7 (3 plus Zn)	25.70	1.37	2.31
8 (4 plus Zn)	25.50	1.38	2.44
Experiment 2—56 days			
1 (Basal)	13.25	1.04	2.20
2 (1 plus CDS)	13.00	1.15	2.19
3 (1 plus Ca)	13.00	0.91	2.26
4 (3 plus CDS)	13.00	0.82	2.69
5 (1 plus Zn)	12.37	0.99	2.12
6 (2 plus Zn)	13.25	1.13	2.24
7 (3 plus Zn)	12.37	1.05	2.46
8 (4 plus Zn)	13.00	1.05	2.40
9 (3 less 2% fat)	13.00	0.94	2.38

[a] Dried corn distillers solubles.

TABLE 3. RESPONSE OF PIGS TO TREATMENTS IN EXPERIMENT 3—37 DAYS

Treatment	Av. initial wt., lb.	Av. daily gain, lb.	Feed per lb. gain, lb.
1 (Basal)	39.25	1.17	3.45
2 (1+CDS)	37.12	1.24	2.91
3 (1+Ca) [a]	37.62	0.62	4.44
4 (3+CDS) [b]	37.25	0.73	4.00
5 (1+Zn)	37.62	1.08	3.45
6 (2+Zn)	37.75	1.31	2.92
7 (3+Zn)	38.50	1.15	3.55
8 (4+Zn)	39.50	1.36	3.08

[a] Four of the eight pigs on treatment 3 developed parakeratosis.
[b] Three of the eight pigs on treatment 4 developed parakeratosis.

TABLE 5. ANALYSIS OF VARIANCE (EXPERIMENT 3)

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatment	7	4.0281	.5754**
DCDS vs. none	1	.3781	.3766
Ca vs. none	1	.8934	.8934**
Zn vs. none	1	1.2543	1.2543**
DCDS x Ca	1	.0000	.0000
DCDS x Zn	1	.0701	.0701
Ca x Zn	1	1.4281	1.4281**
DCDS x Ca x Zn	1	.0041	.0041
Sex	1	.0373	.0373
Interaction	7	.3473	.0496
Error	48	5.1090	.1064
Total	63	9.5217	.....

** Significant, 1% level.

Experiment 3. The results are summarized in tables 3 and 4. Unlike the two previous experiments, differences were noted among treatments when a corn-soybean oilmeal ration was fed to pigs on concrete floors. The first symptoms appeared among pigs on the high calcium treatment; 50% of them developed parakeratosis. Although not so severe, parakeratosis developed among 40% of the pigs on treatment 4 which contained 1.34% calcium plus 5% solubles. No symptoms were noted in pigs on the other ration treatments which were either low calcium or supplemental zinc. Adding 26.7 ppm of zinc to treatment 7 which contained 1.36% calcium prevented parakeratosis and increased growth rate. Dried corn distillers solubles improved the growth rate of pigs which were fed 0.48% calcium rations, (treatments 2 and 6) and pigs fed a 1.36% calcium ration (treatment 8) when supplemented with zinc.

The treatments which were supplemented with 5% distillers solubles increased rate of gain by 13.36% over the unsupplemented rations. These treatments also required 15.50% less feed.

The statistical analysis revealed that there was a significant difference at the 1% level between high calcium treatments and low calcium treatments; likewise there was a significant difference at the 1% level between those rations supplemented with zinc and those unsupplemented. The analysis did not reveal any interaction between distillers solubles and calcium; likewise there was no interaction between solubles and zinc. However, the interaction between calcium and zinc proved to be highly significant at the 1% level.

After dietary changes were made at the end of 37 days zinc supplementation to those animals having parakeratosis resulted in marked increase in rate of gain and alleviation of skin lesions. After two weeks on the zinc supplemented ration, the lesions began disappearing and at the conclusion of the trial the lesions were completely absent. The animals which had been on a high-calcium ration (1.36%) supplemented with zinc did not develop any symptoms of parakeratosis, indicating the possible storage or carryover of zinc giving them protection during the time this trial was conducted.

TABLE 4. RESULTS OF EXPERIMENT 3 AFTER DIETARY CHANGES WERE MADE—26 DAYS

Pre-treatment	Treatment	Av. initial wt., lb.	Pre-treatment av. daily gain	Av. daily gain, lb.	Pre-treatment feed per lb. gain, lb.	Feed per lb. gain, lb.
1 (Basal)	Basal+Zn	82.75	1.17	1.44	3.45	3.59
2 (1+DCDS)	Basal+DCDS+Zn	83.37	1.24	1.61	2.91	3.50
3 (1+Ca)	Basal+Ca+Zn	61.87	0.62	1.35	4.44	3.86
4 (3+DCDS)	Basal+Ca+Zn+DCDS	63.50	0.73	1.64	4.00	3.33
5 (1+Zn)	Basal	77.50	1.08	1.40	3.45	3.64
6 (2+Zn)	Basal+DCDS	86.37	1.31	1.78	2.92	3.24
7 (3+Zn)	Basal +Ca	81.25	1.15	1.64	3.55	4.11
8 (4+Zn)	Basal+Ca+DCDS	89.87	1.36	1.85	3.08	3.77

Summary

In three experiments, 132 pigs were used to study the occurrence of parakeratosis in pigs fed varying levels of calcium and to determine the value of distillers solubles and zinc as a preventive or cure of the disease.

The feeding of semi-purified rations containing levels of calcium up to 1.3% to pigs confined to metal wire-floored crates produced good rates of gain and feed conversion with no symptoms of parakeratosis. The feeding of a corn-soybean meal ration with the calcium level at 0.50% resulted in good growth; with the calcium level raised to 1.36% it resulted in poor growth and 50% parakeratosis. The animals exhibited symptoms as described by Kernkamp and Ferrin (1953).

The addition of 5% distillers solubles to the 1.34% calcium ration did not improve growth, and 40% of the pigs developed parakeratosis. Adding zinc at a level of 26.7 ppm prevented parakeratosis and improved growth.

Distillers solubles improved growth rate of pigs on low-calcium and high-calcium rations supplemented with zinc. Where symptoms of parakeratosis were present, zinc supplementation resulted in increased growth rate and alleviation of symptoms.

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METABOLITES OF PROGESTERONE AND THEIR PHYSIOLOGICAL SIGNIFICANCE IN THE URINE OF PREGNANT AND NON-PREGNANT SOWS AND GILTS [1]

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THE results of investigations during the past few years have emphasized the role of anatomical and physiological factors as determinants of litter size in swine. Among the first major observations in this respect was that less than two-thirds of the total ova shed resulted in viable young at parturition. As early as the 25th day of gestation 35% of the ova shed at estrus are not represented by viable embryos according to Squiers *et al.* (1953). Lerner *et al.* (1957) endeavored to establish the exact time of the greater proportion of the embryonic deaths and reported that an accurate appraisal of normal and abnormal embryos was impossible prior to the 17th day of gestation. The total embryonic loss observed in gilts slaughtered on the 25th day was 33.63% whereas that observed in gilts slaughtered on the 17th day after mating was 25.1%. Thus, approximately two-thirds of the mortality occurring in swine by the 25th day has occurred prior to the 17th day.

Since Squiers *et al.* (1953) found that about 95% of the ova shed were fertilized, the embryonic loss may be attributed to implanation failures and/or early intra-uterine mortality.

Results of experiments with rats by Bredeck and Mayer (1957) suggested that the reproductive hormones, especially progesterone, play an impotrant role in the regulation of the activity rate of phosphatases and other enzymes. That maintenance of an optimal uterine environment for the development of viable embryos is a function of the reproductive hormones was confirmed by Reddy *et al.* (1958). Exogenous progesterone and estrogen administered in minute therapeutic dosages for a 10-day period during a critical period in early pregnancy was shown to exert a general beneficial effect upon the uterus and to promote conditions conducive to maximum embryonic survival.

It is the purpose of this paper to report the results of a qualitative and quantitative study of the metabolites of progesterone and progesterone-like steroids in the urine of sows, and the relationship of these metabolites to embryonic viability during early pregnancy.

Materials and Methods

The sows and gilts from which the urine specimens were collected were part of the herd maintained by the Swine Breeding Project, United States Department of Agriculture and the Missouri Agricultural Experiment Station and were selected from experimental groups employed in an investigation of factors involved in embryonic death losses during early gestation.

Early morning urine samples were collected from non-pregnant animals and from pregnant animals at various stages of gestation. Total 24-hour urine specimens were also collected from sows confined to a metabolism crate and the analytical results compared with those obtained on early morning urine specimens from these sows on the days immediately preceding or following the 24-hour collection period. The similarity in the results obtained with different animals and the constancy of the relationship between the metabolite concentrations, per liter of urine, of 24-hour and of early morning specimens of the same sow warranted the utilization of the less laborious early morning collections in subsequent experiments.

The pregnant animals were slaughtered on specific days after mating and the reproductive tracts were removed. Data were recorded on the number of corpora lutea present and the number of viable embryos in both horns of the uterus.

The chemical procedure employed for the extraction of the conjugated progesterone metabolite from the urine was essentially that of

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Astwood and Jones (1941) with the additional modifications of purification techniques suggested by Miller and Mayer (1960). The added purification steps are as follows: (1) washing the toluene extract of hydrolyzed urine with 0.1N NaOH saturated with NaCl, and (2) adding bentonite to the washed toluene, stirring for 10 minutes, and then filtering. The non-conjugated metabolite of "progesterone" was extracted and purified by the same procedure with the exception that preliminary hydrolysis of the urine in the presence of acid was omitted.

The pregnanediol and related urinary excretion products were determined by modifying a quantitative colorimetric method described by Goldzieher (1948) which utilized zinc chloride and acetyl chloride as the color-producing reagents. Details of the entire chemical assay procedure will be described in a later publication.

Results

Non-pregnant Sows. A total of 27 urine specimens from 9 different non-pregnant sows were analyzed for conjugated and non-conjugated metabolites of progesterone and chemically related steroids. The average concentration of the conjugated metabolite in the urine of non-pregnant sows was 3.3 mg. per liter of urine; whereas that of the non-conjugated metabolites was 1.7 mg. In most of the 27 samples analyzed the metabolite concentration deviated little from the averages reported. The sum of the conjugated plus non-conjugated metabolites averaged 5.0 mg. per liter. The highest value obtained, 8.1 mg. per liter, was reported for one sow. Subsequent analyses of the urine of this sow yielded values of 5.1, 6.4, and 4.7 mg. The lowest concentration obtained was 2.4 mg. per liter. However, four other specimens of urine from this sow contained 4.9, 5.0, 3.5 and 4.6 mg. of total metabolites per liter.

Pregnant Sows. Forty urine samples from 31 pregnant sows were collected during the 24th to 28th day of gestation and analyzed for the conjugated and non-conjugated urinary metabolites. Space limitations preclude the inclusion of these data individually and the mean values are meaningless because of the extensive variation. The concentration of the

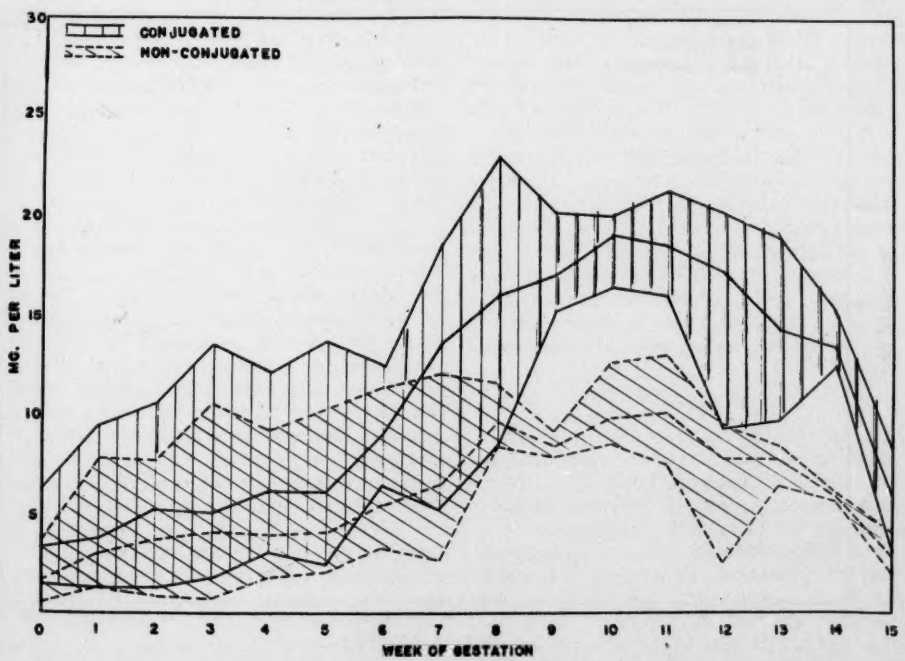

Figure 1. Range and average concentration of metabolites in the urine of the sow during each week of gestation.

conjugated metabolite ranged from 5.0 mg. per liter to 25.0 mg. and the non-conjugated metabolites from 1.8 to 9.0 mg.

The results obtained by quantitative determination of the conjugated and non-conjugated urinary metabolites at weekly intervals throughout 15 weeks of gestation are presented graphically in figure 1. The data represent the average concentration and demonstrate the range in concentration of these metabolites in the urine of the sow during gestation. It will be noted that the conjugated metabolite is present in greater concentration and varies in amount to a greater degree than the non-conjugated metabolites. Further, the markedly high rate of excretion of the conjugated metabolite from the 6th to the 13th week followed by a rapid decrease during the last two weeks of gestation may bear a relationship to the rapid uterine growth due to distention and to endocrine mechanisms governing the initiation of parturition.

The results of the analyses of daily urine specimens from 14 sows during the first 30 days of pregnancy are graphically presented in figure 2. These data show a decreased excretion of urinary metabolites between the 18th to 26th day of gestation. This is the period when an appreciable percentage of the total embryonic mortality occurs as reported by Squiers *et al.* (1953) and Lerner *et al.* (1957).

In figures 1 and 2, it is apparent that there is a close relationship between the excretion rate of the conjugated and non-conjugated metabolites. Statistical analysis shows the correlation coefficient between these to be 0.68 for the entire gestation period, a highly significant figure ($P<.01$).

Correlation of Metabolite Concentration and Certain Factors of Pregnancy. The sows used for the study of the urinary metabolites were those used by Squiers *et al.* (1953) and Lerner *et al.* (1957) on which extensive studies were made of early embryonic mortality. Their research and that reported here were completed during 1949–1953. Correlation coefficients between total urinary metabolite concentration and data obtained in the investigations cited above are presented in table 1.

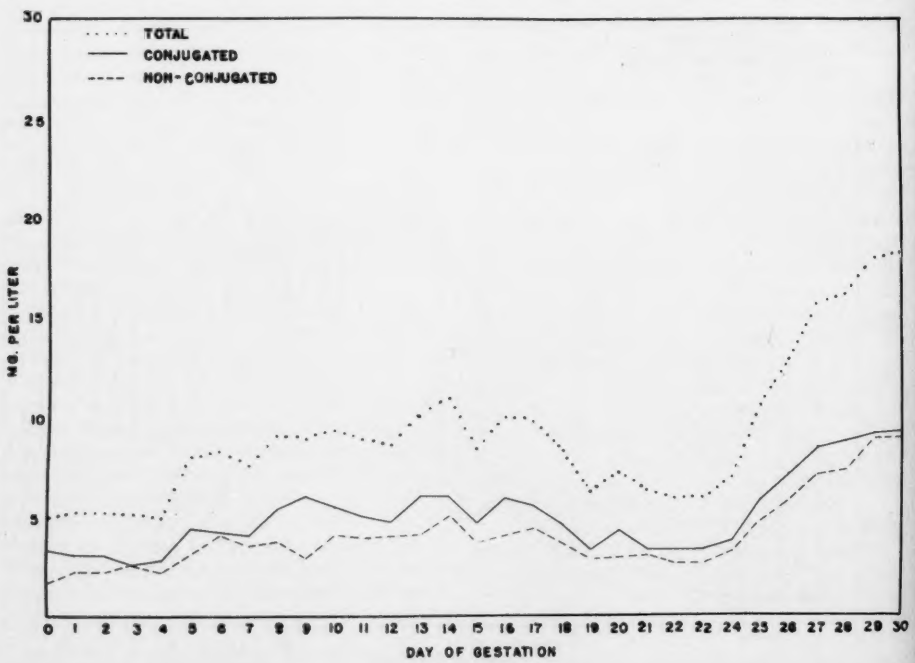

Figure 2. Average concentrations of metabolites in the urine of the sow during the first thirty days of gestation.

TABLE 1. RELATIONSHIP BETWEEN THE CONCENTRATION OF URINARY METABOLITES OF PROGESTERONE AND CERTAIN FACTORS OF PREGNANCY

Item	Correlation coefficient	
	Conjugated	Non-conjugated
A. 16th to 18th day of gestation[a]		
No. of embryos implanted	0.70**	0.65**
Percentage of ova implanted	0.96**	0.62**
No. of corpora lutea	0.56*	0.22
B. 24th to 28th day of gestation[b]		
No. of embryos implanted	0.52**	0.47**
Percentage of ova implanted	0.54**	0.73**
No. of corpora lutea	0.025	0.10

* Significant $P<.05$.
** Significant $P<.01$.
[a] (N=15).
[b] (N=40).

In general, these highly significant correlations indicate the important role of progesterone in the maintenance of embryonic viability. The concentration of the conjugated metabolite is significantly correlated (0.56) with the number of corpora lutea from the 16th to 18th day of gestation, suggesting the corpora lutea as the source of the precursor of this metabolite during this period and perhaps prior to this period. The low correlation coefficients between the number of corpora lutea and the concentration of the non-conjugated fraction during both periods studied and between the corpora number and the concentration of the conjugated fraction from the 24th to the 28th day would suggest that the source of the precursors of these metabolites is some tissue other than the corpora lutea.

Gawienowski and Mayer (1957) reported a significant correlation ($P<.01$) between the amount of progesterone and the number of corpora lutea in the ovaries of sows killed on the 55th day of gestation. Whether this correlation is related specifically to the concentration of the conjugated metabolite remains to be seen by further investigation. Possibly the corpora lutea continue to produce a specific amount of progesterone throughout the sow's pregnancy while other tissues may produce supplemental progestins after the 18th day of pregnancy.

Discussion. The urine of non-pregnant and pregnant sows was analyzed for pregnane derivatives by a modified Astwood and Jones (1941) technique in which the second purification step, precipitation of pregnanediol from alcoholic alkali, was eliminated. Additional purification steps eliminated colored contaminants and chromogenic material and give a final residue which can be quantitatively determined by the colorimetric procedure of Goldzieher (1948).

If the sow urine had been hydrolyzed, as is the usual procedure, only conjugated pregnanediol would have been reported as the metabolite. The observation that the correlation between the concentration of the conjugated fraction and that of the non-conjugated fraction throughout pregnancy was 0.68 is worthy of consideration, in substantiation of the importance of all the reported metabolites to pregnancy.

Smith and Barker (1952) analyzed sodium "pregnanediol" glucuronide crystals from human pregnancy urine and reported that from 2% to 38% of the "pregnanediol" glucuronide complex was accounted for by pregnanolone and some as yet unidentified component of these crystals was associated with the clinical progress of pregnancy in the human female.

Apparently, at different stages in pregnancy the source of the precursors of the urinary metabolites may not be identical. The correlations presented in table 1 indicate that the precursor of the conjugated non-ketonic steroid in the urine was produced by the corpora lutea in early pregnancy and that the placenta and/or other tissue produced this precursor after the 24th day of gestation in the sow.

The precursors of the ketonic and non-ketonic steroids in the non-conjugated fraction apparently were not produced by the corpora lutea from the 15th to 18th day nor during the period from the 24th to 28th day of gestation, as indicated by the low correlation coefficients (table 1). Either the placentae or adrenal glands may produce these precursors, but the results of this investigation give no indication as to their source or identity.

Summary

The urine of non-pregnant and pregnant sows was analyzed for pregnane derivatives by a modified Astwood and Jones technique. The procedure not only determined pregnanediol, which was originally present in the urine in a conjugated form, but also determined a ketonic and a non-ketonic constituent in the non-conjugated fraction. The highly significant correlations reported of the concentration, both of the conjugated and of the two non-conjugated fractions of sow pregnancy urine with the number of embryos implanted and

with the percent of the ova resulting in viable embryos, indicate that all three metabolites are physiologically important in the reproductive processes associated with pregnancy in the sow.

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SWINE METABOLISM UNIT[1]

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IN some nutrition experiments with radioisotopes a metabolism unit must be used which provides for isotope dosing and for quantitative separation of urine and feces. Briggs and Gallup (1949) have described several types of metabolism units for large animals. The design and operation of a swine metabolism unit for radioisotope studies has been reported by Hansard, Plumlee and Comar (1951) of this laboratory. The latter unit suffices for swine weighing 200 ± 50 lb., but not for lighter animals. In the design of a new swine metabolism unit special consideration has been given to collection of excreta, maintenance of animal homeostasis and ease of operation.

Metabolism Unit. The metabolism unit shown in figure 1 consists of a holding box, feed box, a wood-frame supporting structure, and appropriate accessory equipment. Each of these is described below.

The sides of the holding box are made of ¾-inch expanded metal supported by a wood frame made of 2-in. × 4-in. material. The width of the holding box can be varied from 6 to 18 inches by adjusting the sides in or out. To restrain the animal ⅜-inch rods are inserted behind him and bolted to the side grills. A metal restraining grill held by rods is placed over the animal to prevent him from turning a somersault. This grill is shaped to conform with the arch of his back.

A feed box with expanded metal front panel is located at the front of the metabolism unit. A sliding panel for head-width adjustment is mounted between the feed box and the holding box. The feed and water pans are inserted through a small hinged door located in the front of the feed box. The feed pan is secured by two strips of wood to keep the pig from turning it over. The opening between the feed box and the holding box is 4½ inches above the bottom of the holding box. This prevents the pig from resting his feet in the feed pan.

The base of the wood-frame supporting structure is 28 × 40 inches and the height of the structure is 29½ inches. The wood-frame supporting structure was made from planed oak and bolted together as shown in figure 1. The wide base and rigid construction of the supporting unit prevents it from becoming top-heavy when an animal is placed in the holding pen. The animal being elevated makes feeding, bleeding, and collection of excreta convenient.

The accessory components of the metabolism unit shown in figure 2 were designed to conform with the anatomy and temperament of swine. The smooth arched back of barrows and the characteristic jerking while urinating necessitated the use of a 12-inch-long metal grill over the urine pan in lieu of harness and a rubber urine funnel. For rigidity, a wooden frame of 2-in. × 2-in. material was used to enclose the waste feed grill, urine

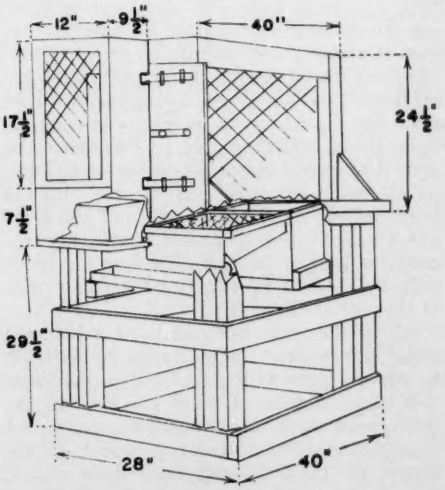

Figure 1. Swine Metabolism Unit.

[1] This manuscript is published with the permission of the Director of the University of Tennessee Agricultural Experiment Station, Knoxville, Tennessee. The work was completed under Contract No. AT-40-1-GEN-242 between the University of Tennessee College of Agriculture and the Atomic Energy Commission.

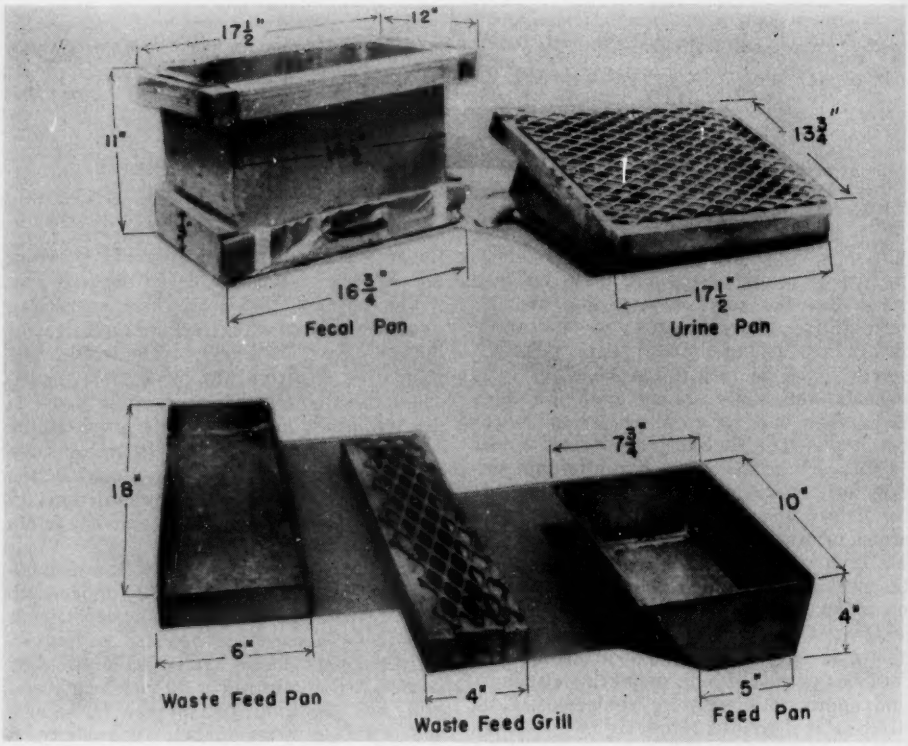

Figure 2. Swine Metabolism Unit—Accessory components.

grill, and fecal pan. The front of the fecal pan was placed immediately behind the rear feet of the pig but slightly in front of the metal rods holding him in the unit. This arrangement was acceptable for fecal collections since the pig backs up as far as possible to defecate. In addition, this trait enabled the animal to have a linear allowance of about 4 inches which permitted him to rest with his head outside the feed box. Behind the fecal pan are 2- × 2-inch spacing boards to complete the floor of the unit. The floor is removable and is held in place by removable pins at the rear of the unit.

All of the pans were made of galvanized metal, but in some studies it may be desirable to replace them with glass-lined or stainless-steel pans. Marine plywood was used in the door, since these units must be washed and decontaminated after each experiment. All the frames for the grills were made from planed oak.

The operation of the metabolism unit is fairly simple. The animals must be very gentle otherwise they may die from struggling against confinement. There should be careful spacing of the urine and feces pans and adjustments made during the trial to collect excreta separately and quantitatively. As discussed by Hansard *et al.* (1951), paper lining was usually used for fecal pans. Presumably gilts could be used in these units as well as barrows. Urine collections could be made with equipment described by Hansard *et al.* (1951).

Bleeding and dosing of the animal is done at the front of the unit. A snare is placed on the snout and the head lifted so that the handle of the snare rests across the rods holding the top. In this position both the mouth and jugular vein are readily accessible.

Summary

A metabolism unit for pigs weighing 100 ±

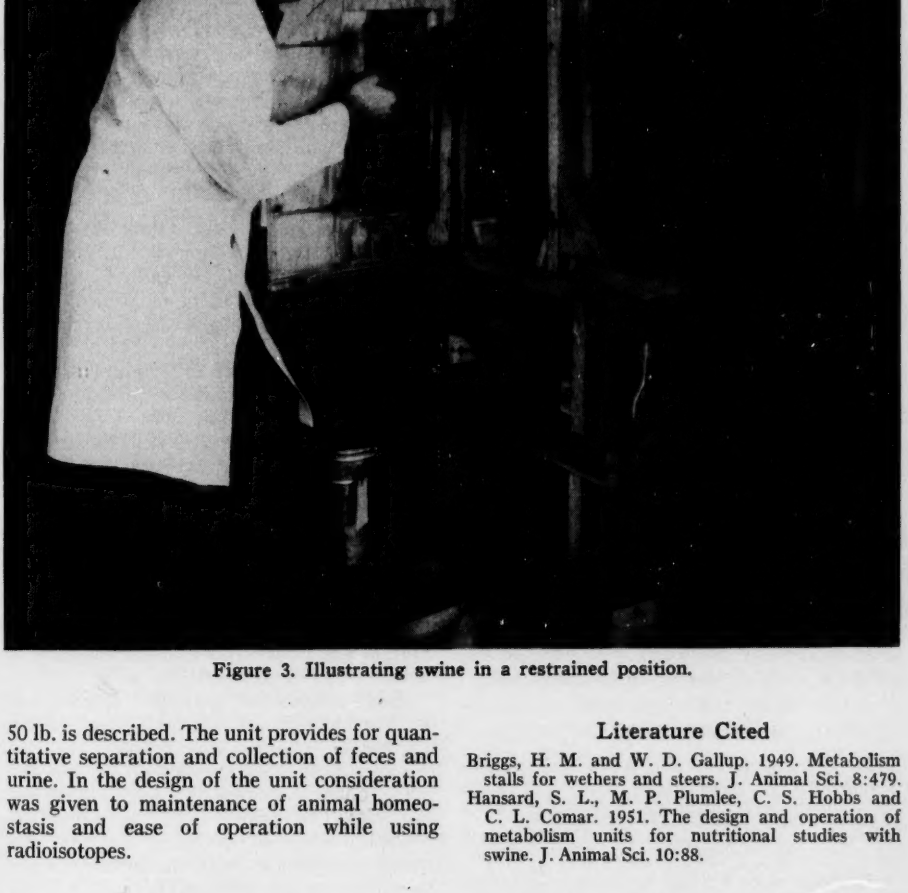

Figure 3. Illustrating swine in a restrained position.

50 lb. is described. The unit provides for quantitative separation and collection of feces and urine. In the design of the unit consideration was given to maintenance of animal homeostasis and ease of operation while using radioisotopes.

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THE FROZEN STORAGE OF RAM SEMEN [1]

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LITTLE research has been conducted with frozen ram semen because of limited artificial insemination in sheep. However, with the possibilities of synchronized estrus and the advent of production and progeny testing, it is possible that artificial insemination will increase. A great saving in reproductive potential could be realized if ram semen were frozen and stored during an entire year. A successful technique for freezing semen could also be a useful tool in genetic research, enabling the use of the semen of ram lambs after slaughter. Frozen ram spermatozoa, to be useful, must survive prolonged frozen storage and be capable of fertilizing ova.

The purpose of these studies was (1) to determine the glycerol requirement for freezing semen, (2) to determine the sperm survival effect of adding egg yolk to a milk diluter and (3) to investigate the rate of decline in motility of frozen ram spermatozoa stored at —79° C. over a 14-month period.

Experimental

In Experiment 1, five ejaculates of one Hampshire ram were diluted with eight levels of glycerol in milk-diluter and frozen. Five trials were involved and the glycerol levels were 0, 2, 4, 6, 8, 10, 12 and 15%.

In Experiment 2, three ejaculates from each of two rams were frozen individually to determine sperm survival after freezing in milk arabinose diluters containing 5, 5.5, 6, 6.5, 7, 7.5, 8 and 8.5% glycerol.

In Experiment 3, involving the pooled ejaculates of two rams in two trials, attempts were made to determine if added egg yolk at levels of 0, 3, 6, 12 or 24% would improve the freezing survival of milk-diluted sperm, and to determine what combination of egg yolk and glycerol levels of 6, 9, 12 and 15% might result in the greatest sperm survival after freezing.

The homogenized milk diluter was heated to 208° F. for 10 minutes; it contained 12.19% solids, 3.45% fat and had a pH of 6.71. Five hundred units of penicillin and 500 micrograms of dihydrostreptomycin were added per ml.

The base diluter used in Experiments 2 and 3 contained 1.25% arabinose in addition to heated homogenized milk.

All semen was collected with an artificial vagina. It was diluted 1:100 at 35° C. The semen was then cooled less than 1° C. per minute to 5° C. except in Experiment 3 where the semen was cooled 3° C. per minute to increase the possibility of temperature shock.

Semen samples after cooling to 5° C. were further diluted with an equal volume of the diluter containing glycerol, such that the final dilution was 1:200. This dilution was made at three 10-minute intervals.

Semen samples in Experiment 1 and 2 were equilibrated with glycerol for 12 to 15 hours, whereas a one-hour equilibration was used in Experiment 3. All samples were frozen as a 1 ml. volume in "Frost T" heat sealed plastic ampoules. The samples to be frozen were suspended in a bath of ethyl alcohol at 5° C. The temperature of the bath was further lowered by adding dry ice to the alcohol so that in Experiment 1 and 2 the temperature dropped 1° C. per minute from 5° to —10° C. then 2° per minute from —10° to —18° C. then 4° per minute from —18° to —35° C. and 5° per minute from —35° to —75° C. The temperature drop was controlled by manually following a graph of this rate with a "Brown" recording potentiometer which recorded every 4.5 seconds.

Samples in Experiment 3 were cooled 3° C. per minute to —15° C. and 5° C. per minute to —75° C. The rapid rate was again used to increase the possibility of temperature shock where egg yolk protection for temperature shock was being tested.

After freezing, the semen was stored under dry ice and alcohol at a temperature of —77°

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C. to —81° C. Semen samples in Experiment 1 were stored one day; 10 months in Experiment 2 and 1 week in Experiment 3.

All semen was thawed in 6° C. to 8° C. water then transferred to an incubation room at 39° C. and held there for 15 minutes before microscopic examination. Slides were prepared by diluting a small wire loopfull of semen in a drop of physiological saline. The slides were examined on a heated stage at 210 × magnification with a Bausch and Lomb binocular phase contrast microscope. Approximately 100 sperm were counted per slide. Two slides were counted per ampoule of semen and at least two ampoules were examined to make up each recorded observation. Percent motility was determined as number of motile sperm counted × 100 divided by total number of sperm counted. Percent survival was determined as motile sperm after freezing × 100 divided by motile sperm before freezing.

In Experiment 4, one ejaculate each from two Hampshire rams was diluted 1:200 and frozen in a heated homogenized milk diluter containing 1.25% arabinose and 7% glycerol. Four vials of semen per ram were periodically thawed and examined for motility.

Results

Ram sperm survival increased as glycerol level increased to 6 or 8% glycerol after which further glycerol addition resulted in lower sperm survival (figure 1). A student range test indicated that the mean sperm survival was significantly greater at 6 and 8% glycerol, than at levels below 6% or above 10% ($P<.05$). There was no significant difference in mean sperm survival between 6 and 8% glycerol or between 8 and 10%.

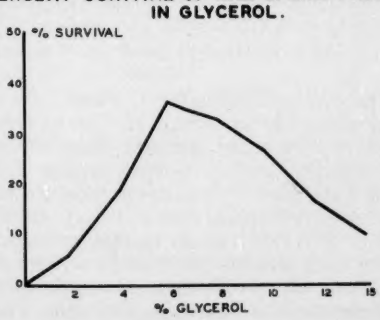

Figure 1.

TABLE 1. MOTILITY OF SPERM AFTER FREEZING AS AFFECTED BY GLYCEROL LEVEL

Glycerol level (%)							
5	5.5	6	6.5	7	7.5	8	8.5
Motility after freezing (%)							
36	39	42*	45	43	42	45	47*

* Significantly more motile sperm than other treatments ($P<.05$).

In Experiment 2, 144 motility observations were used to determine an optimum glycerol level. These were from six thawed frozen ejaculates, split among glycerol levels of 5, 5.5, 6, 6.5, 7, 7.5, 8 and 8.5%.

Estimates of the glycerol level effect, obtained by a least squares analysis with the aid of the electronic computer "MISTIC", indicated significant difference among glycerol levels ($P<.05$). A student range test indicated that sperm motility means after freezing with glycerol levels of 6 to 8.5% were significantly greater than with 5 or 5½% glycerol ($P<.05$). The greater motility, after freezing of the 8.5% level as compared to the 5%, was highly significant ($P<.01$).

The mean percent motility for each glycerol level is shown in table 1.

Hill *et al.* (1959) found an interaction between rams and glycerol levels. None was found in this study. Ample evidence for the reality of such treatment sire interactions have been established for frozen bull semen by White *et al.* (1954), Odell and Hurst (1956) and Hendrikse and Joling (1957).

The survival of ram sperm frozen in a milk diluter with combinations of egg yolk and glycerol, are shown in table 2. These data are averages from the pooled ejaculates of two

TABLE 2. PERCENT SURVIVAL OF RAM SPERM FROZEN IN MILK WITH EGG YOLK AND GLYCEROL AT VARIOUS LEVELS

Glycerol level, 1%	Yolk level, %					
	0	3	6	12	24	Av.
6	71	65	71	55	62	65**
9	85	66	77	67	36	66**
12	49	30	25	15	7	25*
15	1	2	2	0	0	1
Average	51	41	44	34	26	

** The mean is significantly greater than the two lowest means ($P<.01$).

* The means is significantly greater than the lowest mean ($P<.05$).

TABLE 3. ANALYSIS OF VARIANCE ON COMBINATIONS OF YOLK AND GLYCEROL

Source	df	ms	F
Yolk levels	4	1468.20	1.77
Glycerol levels	3	20332.8	24.45**
Yolk and glycerol	12	831.48	9.32**
Trials	1	1.5	0.2
Error	59	89.19	

** Significant ($P<.01$).

rams in two trials. An analysis of the variance in this experiment is presented in table 3.

A highly significant interaction between egg yolk and glycerol levels is evident. This interaction occurred because as yolk increased, sperm survival decreased more rapidly at 9 and 12% glycerol than at 6 or 15%. There is a highly significant difference between glycerol levels when the interaction mean square is used to determine the F ratio. A student range test was applied to test differences among the glycerol level means. Significantly more sperm survived freezing with glycerol levels of 6 or 9% than with levels of 12 or 15% ($P<.01$). The survival mean for 12% glycerol was significantly greater than that for 15% ($P<.05$).

The addition of egg yolk to a heated homogenized milk diluter did not increase ram sperm survival after freezing. The average sperm survival appeared to decrease as egg yolk increased. The sperm survival differences between egg yolk levels, however, were not significant when the interaction mean square was used to determine the F ratio for yolk levels.

The results of Experiment 4 are shown in table 4 and figure 2.

The motility of ram sperm declined rapidly during the first two months of frozen storage after which time little motility decline occurred.

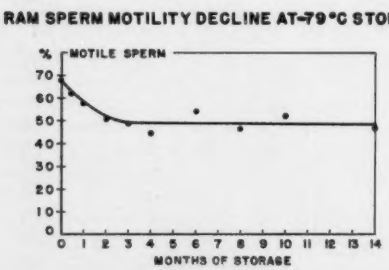

Figure 2.

TABLE 4. PERCENT MOTILITY OF RAM SPERM FROZEN FOR VARIOUS LENGTHS OF TIME

	Rams		
Time after freezing	A	B	Av.
1 hour	67	69	68
2 weeks	62	62	62
1 month	57	61	59
3 months	52	50	51
4 months	47	52	49
6 months	52	56	54
8 months	42	52	47
10 months	49	54	52
14 months	43	50	47

Discussion

The results of experiment 4 are in agreement with the data of Hill *et al.* (1959) wherein frozen ram sperm rapidly lost motility between two days and ten days storage. The results also agree with those of Van Demark *et al.* (1957) who found a progressive decline in frozen bull sperm motility during the first 51 days of storage.

The data of this study do not agree with the increase in motility of stored bull semen reported by Etgen *et al.* (1957) and by Polge and Rowson (1952b), or with Szumowski *et al.* (1956) who found no decline in motility of ram sperm stored for 4 weeks at —79° C.

The studies of Bratton *et al.* (1957), Mixner and Wiggin (1957) and Graham *et al.* (1958) have not demonstrated a decline in first service non-return rate of frozen bull semen when inseminations were performed with semen frozen for various storage intervals.

Data are needed to determine if ram semen stored at —79° C. will maintain its fertilizing capacity through years of storage. First *et al.* (1961) found that 3 of 15 ewe lambs conceived after insemination with thawed ram semen which had been stored for 7 months at —79° C.

The data of Experiment 1, 2 and 3 are all in agreement as far as high and low extremes in glycerol levels are concerned. They indicate the optimum level is between 6 and 10%. From Experiment 2, a single optimum glycerol level does not appear to be sharply defined. Hill *et al.* (1959) found greater survival of sperm after freezing in 3.5, 5.25 or 7% glycerol than in 14%. Szumowski *et al.* (1956) observed no difference in survival after freez-

ing sperm in 6 to 16% glycerol. Emmens and Blackshaw (1950) concluded that 7.5 to 10% glycerol in a yolk phosphate citrate arabinose diluter resulted in the greatest sperm survival after freezing. Since then, White *et al.* (1954), Blackshaw (1955a, b), Emmens and Blackshaw (1955) and Lopyrin and Loginova (1958) have all frozen ram semen in 7.5% glycerol.

The reasons for a rapid reduction in sperm survival with glycerol levels above 10% certainly need further investigation. It is recognized that bull sperm tolerate glycerol levels much higher than 10% even though the optimum for most bull semen diluters is between 7 and 12% (Smith and Polge, 1950; Polge and Rowson, 1953; Emmens and Blackshaw, 1956).

The addition of egg yolk to a heated homogenized milk diluter did not increase ram sperm survival after freezing and sperm survival appeared to decrease after freezing as the yolk level increased. The milk diluter apparently provided sufficient temperature shock protection during rapid cooling, without egg yolk fortification. Hendrikse and Joling (1957) have shown that the motility of bull sperm is reduced if the diluter contains more than 15% egg yolk. However, Sikes and Merilan (1958) found unfrozen bull sperm maintained motility longer if egg yolk were added to a skimmilk diluter.

Amann and Almquist (1957) and Saroff and Mixner (1955) discovered that higher levels of solids or of egg yolk, increase the amounts of glycerol required for the best freezing survival of bull sperm.

Summary and Conclusions

Fifteen ejaculates of ram semen were used in three experiments to study the glycerol requirement for freezing ram semen in a milk diluter. Egg yolk added to a milk diluter for temperature shock protection before freezing was also investigated.

The mean sperm survival after freezing ram semen with 0, 2, 4, 6, 8, 10, 12 and 15% glycerol was significantly greater at 6 and 8% glycerol than below 6% or above 10%.

A further study at glycerol levels of 5, 5.5, 6, 6.5, 7, 7.5, 8 and 8.5% indicated no significant difference in motility after freezing between levels of 6 to 8.5%.

From a study of combinations of egg yolk and glycerol levels, significantly more sperm survived freezing with glycerol levels of 6 or 9% than with 12 or 15%. The addition of egg yolk to a heated homogenized milk diluter did not increase ram sperm survival after freezing. Furthermore, the average sperm survival appeared to decrease as egg yolk increased.

Frozen semen from each of two Hampshire rams was periodically thawed and examined for motility during a 14-month period of —79° C. storage. Motility declined rapidly during the first two months of storage but changed very little thereafter.

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FERTILITY OF FROZEN AND UNFROZEN RAM SEMEN [1]

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PREVIOUS research has shown that a high percentage of ram sperm survives freezing. The successful application of frozen ram semen to sheep breeding depends on the fertility achieved after insemination of thawed, frozen semen. The fertility reported thus far has been low.

Smirnov (1951) achieved 42% conception after inseminating ewes with thawed frozen ram semen. Graca (1955) reported 31.2% conception, Emmens and Blackshaw (1955) reported 5%, and Kuznetsov (1956) reported 19.3% and 33.5% in two different studies.

This paper presents the data of five breeding trials conducted to study the fertility of frozen ram semen.

Experimental

Ram semen was collected with either an artificial vagina or by means of electrical stimulation. Dilution, freezing and microscopic examination were according to the procedures of First *et al.* (1961).

For insemination, in experiments 1 through 4, the rear quarters of ewes in estrus were elevated in a breeding crate. The cervix was located with the aid of a head lamp and a glass or a spreading speculum. Semen was then deposited in the cervix with a 2 ml. syringe and a drawn glass pipette. In experiment 5 ram semen was deposited in the anterior vagina near the cervix. All ewes were inseminated at one estrus only.

Experiment 1: In the first experiment each of 12 Hampshire ewes was cervically inseminated with 500 million sperm in 1.0 ml. of milk-diluted, thawed, frozen ram semen. Each of 11 other Hampshire ewes was cervically inseminated with 600 million sperm in 0.2 ml. of undiluted ram semen. All ewes were checked for estrus at 12-hour intervals with an aproned ram. Each ewe was inseminated approximately 12 hours after the observed beginning of standing estrus. The frozen and unfrozen semen were obtained from the same Hampshire ram. The frozen semen was collected and frozen two weeks before breeding began. The semen averaged 64% motile sperm before freezing and 15% after freezing. The frozen semen was diluted 1:6. The heated homogenized milk diluter contained 7% glycerol and 1.25% arabinose, plus 500 units penicillin and 500 micrograms dihydrostreptomycin per ml. Table 1 presents the results of this experiment.

Experiment 2: Ten Hampshire ewes were each cervically inseminated with 1.0 ml. of frozen semen from the same Hampshire ram that was used in experiment 1. Ten other Hampshire ewes were each cervically inseminated with 0.2 ml. of fresh, undiluted semen from the same ram. The semen used for freezing contained 3.2 billion sperm per ml. before dilution, of which 80% were motile. The semen was diluted 1:15 with heated homogenized milk; this diluter contained 2% egg yolk, 7% glycerol and 1.25% arabinose, plus 500 units penicillin and 500 micrograms streptomycin per ml. After freezing, 38% of the sperm were motile. Frozen semen was stored at −79° C. for two weeks before breeding began. The unfrozen undiluted semen was inseminated immediately after collection. The insemination dose for unfrozen semen was 0.2 ml.; 80% of the sperm were motile at the time of insemination.

All ewes were checked for estrus at 12-hour intervals with an aproned ram. Each ewe was repeatedly inseminated at 12-hour intervals for as long as she remained in estrus.

Semen quality at insemination and the results of this experiment are presented in table 1.

Experiment 3: After completing experiments 1 and 2, it seemed evident that several factors relating to the insemination of frozen ram semen needed investigation and that an accurate comparison of various treatments could be obtained only if all inseminations contained a constant number of sperm. It also seemed advisable to provide a control of unfrozen diluted semen. Experiment 3, therefore,

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TABLE 1. PERCENT EWES LAMBING FROM INSEMINATION WITH FROZEN AND UNFROZEN SEMEN

Semen	Motility at insem. %	Total sperm insem.	No. ewes	Percent lambing first service	Percent lamb crop
Experiment 1					
Frozen	15	0.500[a]	12	17	33
Unfrozen	80	.600	11	64	108
Experiment 2					
Frozen	38	.213	10	30	50
Unfrozen	80	.600	10	60	80

[a] Billion sperm.

was designed to compare the fertility of inseminated frozen semen with unfrozen semen, both of which were diluted with three different diluters and used in 0.5-ml. and 1.0-ml. volumes.

A factorial experiment was designed in which the semen of seven Hampshire ram lambs and one Shropshire lamb was used to inseminate 96 mature white-faced western ewes. Ewes were allotted to each sire after balancing for weight and previous breeding history. Within a sire group 12 ewes were randomly allocated to each of the following insemination treatments: (1) frozen semen diluted in yolk-citrate and inseminated as 0.5 ml. containing 100 million sperm; (2) a like treatment but with the 100 million sperm inseminated in 1.0 ml. of extender; (3) semen frozen in milk and inseminated as 0.5 ml.; (4) semen frozen in milk and inseminated as 1.0 ml.; (5) semen frozen in yolk-citrate-glucose-urea diluter and inseminated as 100 million sperm in 0.5 ml. of extender; (6) semen frozen in the same yolk-citrate-glucose-urea diluter but inseminated as 100 million sperm in 1.0 ml. of extender. A total of six unfrozen treatments identical to the previous six frozen treatments was used.

Unfrozen semen was glycerolated and handled in all ways except freezing exactly like its frozen counterpart. The frozen semen was collected, frozen and stored 2 weeks before the actual insemination began.

Semen for unfrozen semen insemination was collected from the rams every second day, diluted, examined, and stored in vials at 5° C. This allowed ewes to be inseminated with 8-, 20-, 32- or 44-hour old semen.

The ewes were checked for estrus at 12-hour intervals by exposing them to aproned rams. They were cervically inseminated 12 hours after estrus was observed. Unfrozen semen and thawed, frozen semen were inseminated at a temperature of 5° C.

The yolk-citrate diluter contained 2.1% sodium citrate, 7% glycerol and 1.25% arabinose. The milk diluter contained 7% glycerol and 1.25% arabinose added to a heated homogenized milk of 12% total solids and 3.45% fat. The yolk-citrate-glucose-urea diluter contained 15.8% egg yolk, 2.6% sodium citrate, 6.5% glycerol, 0.65% glucose and 0.15% urea.

The quality of the frozen and unfrozen semen inseminated in this study is shown in table 2.

Sixteen ejaculates from eight rams comprise the semen that was frozen. The same eight rams provided the unfrozen semen that was inseminated. In all, 64 ejaculates of unfrozen semen were used.

Three of the 96 ewes did not come in heat. Therefore, unequal numbers were inseminated with the semen of three diluter and two insemination volumes.

The lambing results of this experiment are shown in table 3.

None of the 47 ewes inseminated with frozen semen lambed even though each in-

TABLE 2. MEASURES OF SEMEN QUALITY

				Before freezing		1 wk. after freezing	
Semen	No. of ejaculates	Vol. (ml.)	Billion per ml.	Progressive motility (%)	Motile sperm (%)	Progressive motility (%)	Motile sperm (%)
Frozen	16	0.99	3.91	74.6	88.2	58.3	69.6
Unfrozen	64	0.99	4.46	71.3	85.5	....	....

TABLE 3. PERCENT OF EWES LAMBING FROM INSEMINATION WITH UNFROZEN AND FROZEN SEMEN

Diluter	Frozen semen		Unfrozen semen	
	No. ewes bred	Percent conception	No. ewes bred	Percent conception
Yolk-citrate	16	0	14	21
Milk	16	0	15	47
YCGU[a]	15	0	17	18
Volume				
1.0 ml.	24	0	22	23
0.5 ml.	23	0	24	33

[a] Yolk-citrate-glucose-urea.

semination contained nearly 70 million live sperm.

Frozen semen samples, identical with those used for insemination, were examined for motility 4 months after freezing. Sixty-two percent of the thawed, frozen sperm diluted with milk, 68% of the sperm diluted with yolk-citrate and 68% of the sperm diluted with the Y-C-G-U diluter were motile.

Inseminating unfrozen semen stored at 5° C. gave the following results: 21% of the ewes lambed when inseminated with 100 million sperm diluted in yolk-citrate; 47% with milk-diluted semen; and, 18% with semen in the Y-C-G-U diluter.

The milk diluter appeared superior. However, the F value of 1.91 for diluters in table 4 indicated no significant difference existed among the three diluters ($P>.05$).

Thirty-three percent of the ewes lambed when inseminated with 100 million sperm in 0.5 ml. of fluid, and 23% lambed after the insemination of 100 million sperm in 1.0 ml. of fluid.

The F value of 0.6 for volume effect in table 4 indicated that the differences in lambing rate due to volume of semen inseminated were not significant ($P>.05$). The number of ewes bred with semen of 8 to 44 hours of age and the number of ewes lambing from insemination of semen of various ages is shown in table 5.

TABLE 4. ANALYSIS OF VARIANCE AMONG EWES CONCEIVING FROM UNFROZEN SEMEN

Source	df	ms	f
Diluters	2	.375	1.91
Volume	1	.120	.61
Diluters x volume	2	.293	1.50
Error	40	.196	....

There were too few observations to determine accurately whether the three diluters responded differently after different lengths of storage. The combined data of the three diluters in table 5 do suggest that up to 44 hours semen storage at 5° C. had little effect on lambing rate after insemination.

Cervical mucus was observed in the vagina at the time of insemination in 4 of the 13 ewes conceiving, and in 76% of the 93 ewes inseminated. Sinclair (1957) identified the physiological mid-estrus by a clear and copious cervical mucus.

Experiment 4: A fourth breeding experiment was designed to study, first of all, the effect of one frozen semen insemination during estrus as compared with insemination every 12 hours, and secondly, to determine if vasectomized mating immediately before frozen semen insemination might improve conception. To study these factors, 32 ewe lambs were cervically inseminated with milk-diluted frozen semen obtained from two Hampshire rams.

TABLE 5. NUMBER OF EWES LAMBING AFTER INSEMINATION WITH RAM SEMEN OF VARIOUS AGES

Age of semen at insemination	Less than 8 hours	20 hours	32 hours	44 hours
No. ewes inseminated	14	14	4	14
No. ewes lambing	5	3	1	4

An equal number of ewes were inseminated with the frozen semen from each ram. One-half of the frozen semen had been stored 7 months at —79° C. before insemination and half stored 3 weeks. At the time of insemination, 1.0 ml. of the 7-month semen contained 208 million sperm, 36% of these being motile. A ml. insemination dose of three-week stored semen contained 150 million sperm, 60% of these being motile.

The results of this experiment are shown in table 6.

There was no difference in lambing rate between ewes inseminated 12 hours after the onset of estrus and ewes inseminated every 12 hours throughout estrus. In each case, 12.5% of the ewes lambed. Lambing rate was not increased or decreased by allowing a vasectomized mating before insemination. Only 4 of the 32 lambs, lambed.

Experiment 5: The purpose of this experiment was to compare conception rates from

TABLE 6. PERCENT OF EWES LAMBING IN EXPERIMENT 4

Item	No. of ewes	Percent lambing
One insemination	16	12.5
Inseminated every 12 hours	16	12.5
Vasectomized mating	16	12.5
No vasectomized mating	16	12.5
Frozen 7 months (36%—208M)	15	20.0
Frozen 3 weeks (60%—150M)	17	5.8

the insemination of frozen and unfrozen semen and to determine if motility stimulation through saline redilution might increase conception rates.

Thirty-six mature Merino X Columbia crossbred ewes were vaginally inseminated with semen from three Dorset rams. Each ewe was inseminated once, 12 hours after the first observed standing estrus. A standard insemination dose of 300 million sperm in 0.8 ml. of heated, homogenized milk-glycerol-arabinose diluter was used.

Eighteen ewes were inseminated with frozen semen stored at —79° C. and 18 with unfrozen semen stored for less than 2 days at 5° C. One-half of the ewes in each of these groups were inseminated with 0.5 ml. of milk-diluted frozen and unfrozen semen which was diluted to 0.8 ml. with mamalian Ringer's solution before insemination. The remaining one-half were inseminated with 0.5 ml. of milk-diluted semen which was diluted to 0.8 ml. with milk diluter immediately prior to insemination.

Of the sperm to be frozen, 65% were motile before freezing and 30% were motile after freezing and —79° C. storage. The semen used for unfrozen inseminations averaged 78% sperm motility. Four ewes were lost from the experiment before lambing. The number in each treatment at lambing time, therefore were not equal.

The results of this experiment are shown in table 7.

TABLE 7. RESPONSE OF EWES TO INSEMINATIONS IN EXPERIMENT 5

Treatment	Frozen	Unfrozen	Non-stimulated	Saline stimulated
No. inseminated	17	15	17	15
Ewes lambing, no.	4	12	10	8
Lambing, %	23	80	58	53
No. of lambs	5	17	11	11
Lamb crop, %	29	113	64	73

Significantly more ewes lambed from the insemination of unfrozen semen than from the insemination of frozen semen ($P>.01$). Saline motility stimulation had no significant effect on lambing percent.

Discussion

Lambing rates of 17% in experiment 1, 30% in experiment 2, 12.5% in experiment 4 and 23% in experiment 5, compare favorably with the conceptions or non-returns from the insemination of frozen semen reported by other authors. The fact that in experiment 3 none of the ewes inseminated with frozen semen conceived is puzzling in view of the high motility of the semen and especially after some fertility success in the other trials.

The conception rate for unfrozen semen inseminated in experiment 3 seems low in terms of the results of experiment 5 and reports of others. Aslanjan (1950) obtained 92% conception from semen diluted and stored up to 7 days in yolk-citrate. Duzier *et al.* (1954) found conceptions were low from yolk-citrate diluted semen unless the semen was used soon after collection. Istvan (1956) and Dauzier (1956) have achieved higher conception rates with milk diluents than yolk-citrate. Fillimon *et al.* (1956) and Mihailov (1957) have also found milk to be a satisfactory semen diluter, Mihailov (1957) reporting conception rates of 85 to 97% on 4,700 ewes.

Hendrikse and Joling (1957) observed that head agglutination of bull sperm occurred more frequently in citrate-diluted semen than in milk-diluted semen. No particular agglutination differences were observed between any of the diluters studied here, although occasionally some agglutination occurred with each diluter.

The 100 million total number of sperm inseminated was considered sufficient since Kuznecov (1934), Keast and Morley (1949), and Koger (1951) had reported satisfactory conception rates from insemination of 50 million sperm. More than 100 million sperm may be required, however, when the semen has been frozen or diluted and stored at 5° C.

In experiment 4, the 4 ewes lambing, of a total of 32 ewes inseminated, are too few to allow final conclusions. However, one insemination during estrus was as satisfactory as several inseminations. Quinlan *et al.* (1952), Peregon (1936), as cited by Anderson (1945), Avramov (1937), Anderson (1941), Larrea

(1944), Glembockii and Vasiljev (1944), Gutierriez (1948) and Aamdal and Hogset (1955) have all reported slightly increased conception rates from two inseminations during one estrus as compared to one. However, Dauzier *et al.* (1954) and Lopyrin and Loginova (1939) could find no advantage of two inseminations over one.

Summary and Conclusions

Five breeding experiments were involved in a study of the fertilizing ability of frozen and unfrozen semen. In the first experiment, each of 12 ewes was cervically inseminated with 75 million live sperm in 1.0 ml. of milk-diluted, thawed, frozen semen, and each of 11 control ewes was inseminated with 480 million live sperm in 0.2 ml. of undiluted semen. Seventeen percent of the ewes inseminated with thawed, frozen semen lambed as compared to 64% of the control ewes.

In a second experiment, each of 10 ewes was cervically inseminated with 81 million live sperm in 1.0 ml. of yolk-milk diluted, thawed, frozen semen, and each of 10 control ewes was inseminated with 480 million live sperm in 0.2 ml. of undiluted semen. Thirty percent of the ewes inseminated with thawed, frozen semen lambed as compared to 60% of the control ewes.

In a third experiment, none of 47 ewes conceived when cervically inseminated with 100 million thawed, frozen sperm contained in 1.0 ml. or 0.5 ml. of semen diluted in heated homogenized milk, yolk-citrate or yolk-citrate-glucose-urea diluter. The average motility of thawed semen one week after freezing was 70%. Forty-six ewes were cervically inseminated with 100 million unfrozen sperm diluted in the above three diluters. Eighty-six percent of the sperm were motile. The percent of ewes lambing after insemination of semen diluted in the three diluters was, yolk-citrate, 21.4%; milk, 46.7%; and Y-C-G-U, 17.7%. Thirty-three percent of 24 ewes inseminated with 0.5 ml. of diluted semen lambed, as compared with 23% of 22 ewes inseminated with 1.0 ml. Analysis of variance indicated no significant difference among diluters or volumes.

In a fourth experiment, 32 ewe lambs were each cervically inseminated with 1.0 ml. of milk-diluted thawed, frozen semen. No difference in lambing rate occurred between ewes inseminated once during estrus and those inseminated at 12-hour intervals throughout estrus. In each case, 12.5% of the ewes lambed. No difference in lambing rate occurred between ewes that were mated to a vasectomized ram before insemination and ewes that were not. In each case, 12% lambed.

In a fifth experiment, 4 of 17 ewes inseminated with frozen semen lambed and 12 of 15 ewes inseminated with milk-diluted unfrozen semen lambed. Motility stimulation by saline addition before insemination did not increase the lambing rate.

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BLOAT IN CATTLE. I. EFFECT OF BOVINE SALIVA AND PLANT MUCIN ON FROTHING RUMEN CONTENTS IN ALFALFA BLOAT[1]

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SALIVA has been suggested as being a factor in bloat; however, its role has been controversial. Johns (1958) considered that large saliva secretion could either assist in preventing bloat by buffering a fall in pH or increase bloat severity by adding to the CO_2 evolved and by assisting in foam formation. He also believed that mucoproteins present in saliva help form a stable, viscous-type foam. Phillipson and Reid (1958) found that pressure in the rumen caused (1) an increase in the rate of secretion of the parotid and submaxillary glands of some animals and (2) an increase in the level of mucoprotein in the submaxillary saliva. Results of their experiments suggested that additional saliva flow in response to pressure in the rumen may cause increased bloating. Mangan (1958) found salivary mucoprotein an efficient foaming agent *in vitro* but its greatest foam strength was at a pH above that normally found in the rumen.

Weiss (1953) postulated that saliva played a beneficial role in bloat. Working with Merino sheep, he found formation of froth dependent on the consistency of the ruminal ingesta. Bloat caused by frothing of thick, viscid, ruminal ingesta occurred immediately after succulent, leafy legume was fed. When mature stalky legume was fed, the ruminal ingesta reverted to a watery consistency and bloat ceased. Weiss concluded that when succulent legume is eaten, frothy bloat results from insufficient salivation. Bartley (1958) observed that identical twins bloated less with a watery ingesta than with a thick viscid ingesta. Since saliva had been indicated to affect the consistency of the ingesta and, it being a source of mucin, the effect of mucin in preventing bloat was tested. Using linseed meal as a source of mucin, Bartley (1957) found the number of bloat cases not greatly reduced immediately but, after experimental animals were in dry lot 2 to 5 hours, the number of bloat cases was fewer and the bloat index was lower among those fed linseed meal than among the controls.

The work reported in this paper was done to furnish more information concerning the effect of saliva and linseed meal mucin on frothing rumen contents *in vitro*.

Methods

Saliva was collected from fistulated animals. The rumen was emptied and a tube was placed through it, through the cardia and into the posterior esophagus. Saliva was then drawn with a light vacuum into an aspirator flask.

Linseed meal mucin was prepared by Mason and Hall's (1948) water extraction method with minor alterations. One hundred gm. linseed meal was added, with vigorous agitation, to 4 liters of water at 60° C. containing 20 gm. sodium chloride and buffered to a pH 7.0 with sodium bicarbonate. The solution was maintained at 60° C. about 1 hour and then allowed to stand overnight at room temperature. The supernatant was then decanted and used as the linseed meal mucin solution.

The saliva contained an average of 0.011 gm. solids/ml. and the linseed meal mucin solution, 0.017 gm. solids/ml.

Rumen contents were collected from fistulated animals for *in vitro* gas production trials. Approximately 5 kg. of rumen contents were collected in a bucket after the rumen contents had been mixed by hand for approximately 3 minutes. This material was representative of the ingested feed, liquid and froth present in the rumen. In bloated animals some frothy ingesta would blow out through the cannula before the rumen contents could be removed. After mixing, 200-gm. samples were weighed into quart jars, stoppered and placed in a water bath at 39° C. Bovine saliva or linseed meal mucin solution was

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added at different levels to all jars except controls. Rubber tubing connected the jars to calibrated cylinders containing water at room temperature to measure the amount of gas released from rumen contents by water displacement. Readings were taken at 15, 30, 45, and 60 min. In the first trials levels of 20, 80, and 160 ml. of saliva or linseed meal mucin solution were added to frothing ingesta from bloated animals on alfalfa pasture. Trials were then repeated under the same conditions except that the 160-ml. level was omitted. Gas analysis trials were also conducted using nonfrothing rumen contents, to determine if adding 80 ml. of saliva would affect the amount of gas released.

Results and Discussion

Amounts of gas evolved from 200 gm. samples of frothing rumen contents to which 20, 80, and 160 ml. of saliva or linseed meal mucin solution had been added are shown in table 1. Adding 160 ml. of saliva or mucin released less gas than did adding 80 ml. but more than adding 20 ml. Gas release values for the repeated trials using only 20 and 80 ml. of saliva or mucin solution are shown in table 2. The values listed for each time represent the mean of 12 trials. In the 12 trials, without exception, more gas escaped from the frothing rumen samples containing saliva or mucin than from controls. Differences were significant ($P<0.01$) as shown in table 2. In the 60-min. test, 80-ml. levels of saliva or of mucin each released significantly more trapped gas than was released from the control samples. The 20-ml. levels were less effective with differences only approaching significance.

In two trials for which data are not shown, water at 39° C. was added to frothing rumen contents to see if the physical action involved by adding a liquid affected gas release.

TABLE 1. EFFECT OF DIFFERENT LEVELS OF SALIVA AND LINSEED MEAL MUCIN SOLUTION ON THE RELEASE OF TRAPPED GAS FROM FROTHING RUMEN CONTENTS

Treatment	No. trials	Gas released from 200 gm. rumen contents (ml.) 15 min.	30 min.	45 min.	60 min.
Control	2	56	92	136	168
20 ml. mucin	2	52	96	142	184
20 ml. saliva	2	66	112	158	195
80 ml. mucin	2	62	113	169	215
80 ml. saliva	2	80	138	192	232
160 ml. mucin	2	66	106	160	201
160 ml. saliva	2	74	110	161	204

TABLE 2. EFFECT OF SALIVA AND LINSEED MEAL MUCIN SOLUTION ON RELEASE OF GAS FROM FROTHING RUMEN CONTENTS AND THE EFFECT OF SALIVA ON THE PRODUCTION OF GAS BY NONFROTHING RUMEN CONTENTS

Treatment	No. trials	Gas released from 200 gm. rumen contents (ml.) 15 min.	30 min.	45 min.	60 min.
Frothing rumen contents					
Control	12	51	93	129	150
20 ml. mucin	12	58**	105**	147**	175**
20 ml. saliva	12	62**	107**	146**	171**
80 ml. mucin	12	68**	124**	174**	197**
80 ml. saliva	12	71**	129**	177**	202**
Nonfrothing rumen contents					
Control	4	20	32	65	115
80 ml. saliva	4	19	30	64	121

** Significant at the 1% level.

Samples to which water had been added released slightly less gas than samples without additives.

The gas analysis trials with nonfrothing rumen contents (table 2) showed that adding saliva did not cause more gas to be released than was released from the controls. Gas released from froth is from two sources, that produced by the microorganisms during incubation and gas previously produced and trapped in the rumen froth. Since saliva added to nonfrothing rumen contents had no effect, it may be assumed that saliva and linseed meal mucin solution added to frothing rumen samples increased the quantity of collected gas by permitting gas already trapped in the froth to escape rather than by forming new gas.

Work reported here indicates that the reason bloated animals fed linseed meal recovered more rapidly than control animals (Bartley, 1957) may have been that linseed meal mucin causes gas trapped in froth to be released. The effect of saliva on the release of gas trapped in frothing rumen contents also supports the proposal of Weiss (1953) that reduced salivation is a factor in bloat etiology.

If saliva or some constituent of saliva were the only factor involved in bloat production, bloat would be produced when pasturing succulent grass the same as when pasturing succulent legumes. Frothy bloat is apparently stimulated by compounds that augment froth formation, but many of the compounds believed to promote frothing in the rumen are also present in dry forage in nearly the same concentrations as in the green forage. Bloat, then, must result from the presence of froth promoting compounds with an absence of an antifrothing factor. Postulation from the

results of this study is that mucin in saliva may be the antifrothing agent necessary to prevent bloat. However, further studies concerning the nature of the mucin compound involved are needed.

Summary

The effect of saliva and linseed meal mucin solution on the release of trapped gas from frothing rumen contents was tested *in vitro*. The addition of either 80 ml. of saliva or linseed meal mucin solution was found to increase the rate of release of trapped gas from 200 gm. samples of frothing rumen contents. Hence it appears that reduced salivation is a factor in bloat etiology.

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ERADICATION OF ATROPHIC RHINITIS AND VIRUS PIG PNEUMONIA FROM A SWINE HERD

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THE effect of atrophic rhinitis (AR) and virus pig pneumonia (VPP) on the performance of swine has been demonstrated several times. Kristjansson and Gwatkin (1955), Shuman and Earl (1956) and Elliott (1957) reported reduced growth rate among pigs affected with AR as compared to non-affected pigs in the same herd. Betts *et al.* (1955b) observed a 16% reduction in growth rate and a 22% reduction in efficiency of feed utilization in pigs affected with VPP. Betts and Beveridge (1953) estimated that unaffected pigs gain approximately 25% faster than VPP-affected pigs. Young *et al.* (1959) found in a group of 213 slaughter hogs that the incidence and severity of VPP was inversely related to growth rate. Examination of the snouts from these pigs, however, failed to reveal an effect of AR on growth rate, contradictory to the findings of others.

Young and Underdahl (1956) estimated that 40 to 60% of U.S. swine are affected with VPP. VPP lesions were observed in 74% of the hogs examined in a U. S. meat-packing plant by Betts (1956). The incidence of AR in U. S. swine has not been studied extensively, but recent concern among swine producers over this disease reflects a widespread occurrence.

Programs for the eradication of VPP from commercial swine herds have been described by Betts (1952), Betts *et al.* (1955a), Whittlestone and Betts (1955) and MacPherson and Shanks (1955) based on breaking the cycle of infection by isolating each farrowing sow. Only those sows that produced litters showing no signs of VPP were maintained in the herd. The presence of affected pigs in a given litter indicated that infection came from the sow. Johnson *et al.* (1955) reported that atrophic rhinitis was prevented by avoiding all contact of newborn pigs with the sow except that they were allowed to nurse the disinfected udder at intervals during the first 24 hours after birth.

Whitehair and Thompson (1956) described a technique for raising pigs taken by caesarean section and not given colostrum. Elaborate techniques have been described for obtaining pigs by hysterectomy (Young *et al.*, 1955; Underdahl and Young, 1957a) and for raising them in special brooders under sterile conditions (Young and Underdahl, 1951, 1953; Underdahl and Young, 1957b). Repopulation of farms with AR-free, VPP-free ("disease free") swine obtained in this way has recently been reported (Young and Caldwell, 1958).

The high incidence of AR and VPP observed in the Cornell University swine herd in recent years prompted the need for an eradication program. This report describes the procedure used and the preliminary results obtained.

Procedure

The sows from which pigs were to be obtained for establishing an AR-free, VPP-free herd were taken from the swine farm on the 112th day of gestation to clean farrowing crates located in a building where swine had not previously been kept. Twenty-four-hour attendance was maintained until farrowing was completed. As each pig was delivered he was quickly placed in a clean, not previously used wooden box and immediately taken to another building equipped with electric poultry brooders for individual housing at 85° F.

No food was provided for the first 12 hours. One ml. of an antibiotic preparation [3] and 15 ml. of porcine gamma globulin [4] or 10 ml. of anti-hog cholera serum [4] were injected intra-

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[3] Supplied by Upjohn Laboratory, Kalamazoo, Mich. Penicillin-streptomycin mixture supplied as Combiotic.

[4] Supplied by Armour Pharmaceutical Co., Kankakee, Ill. Anti-hog cholera serum supplied as Antrate.

TABLE 1. SURVIVAL RATE OF PIGS RAISED "ARTIFICIALLY"

Litter no.[a]	Litter size (no. of pigs)			
	Raised	Died	Stillborn	Total farrowed
1	4	1	0	5
2	8	3[b]	2	13
3	7	5[b]	2	14
4	6	0	0	6
5	5	2	0	7
6	1	2[c]	2	5
7	1	10[c]	0	11
8	1	5[c]	0	6
Total	33[d]	28	6	67

[a] All pigs in litters 1–5 were given 15 ml. of porcine gamma globulin intra-peritoneally within 24 hours after birth. All pigs in litters 6–8 were given 10 ml. of anti-hog cholera serum (Antrate) subcutaneously within 24 hours after birth.

[b] All but one of these eight pigs died immediately after an antibiotic injection, probably due to an overdose.

[c] Enteric infections, difficult to control with antibiotics, were prevalent in these litters.

[d] Of 61 pigs born alive 33 were raised successfully (54.1% survival rate).

peritoneally during the first 24 hours (table 1).

Liquid synthetic milk [5] was provided from porcelain bowls four times daily until the pigs reached three weeks of age. The amount provided per feeding was regulated to prevent scouring, but was increased gradually from 25 ml. per feeding the first day to as much as 250 ml. per feeding at three weeks of age. Chlortetracycline [6] was added to the milk at each feeding during the latter half of the liquid milk feeding period to minimize scouring. At three weeks of age the pigs were removed to concrete pens in groups of six and dry feed [7] was introduced. All pigs were immunized against hog cholera and erysipelas at approximately 12 weeks of age and the boar pigs (except three retained for later use) were castrated the following week.

Strict isolation from other pigs was maintained throughout the period to market weight or maturity. Housing was in a newly constructed building approximately 50 yards from the main swine barn, and surrounded by swine-lots. The same personnel fed and cared for the swine in both barns, and feed storage and mixing was done at the main barn. Human traffic through the isolation barn was restricted largely to the labor force. A foot bath containing a cresol disinfectant was kept at the door of the isolation barn.

Six gilts from the "artificially raised" group were selected at about 200 lb. to serve as foundation stock. Two were bred by natural service to a boar raised with them in isolation, and four were bred by artificial insemination to a herd boar. All other pigs raised with them in isolation were slaughtered and their snouts and lungs were checked for AR and VPP, respectively.

The six bred gilts farrowed at approximately one year of age and raised conventionally a total of 39 out of 47 pigs farrowed alive without leaving the isolation barn (five of the eight deaths occurred in a litter of eight in which the sow failed to produce enough milk during the first week). An additional 12 gilts were saved from the six litters so farrowed. These gilts represented the second generation of pigs raised in isolation. The remaining pigs were slaughtered and their snouts and lungs were checked for AR and VPP, respectively, as in the first group. Slaughter hogs being raised concurrently in the main barn were also checked at intervals to provide comparable estimates of the level of infection in the two populations.

Results and Discussion

The survival rate of pigs raised on synthetic milk without colostrum is given in table 1. Thirty-three out of 61 pigs born alive (54.1%) were successfully raised in this way. Scouring was a minor problem in the first five litters, but feed intake was controlled at the expense of rapid gains during the first three weeks to minimize scouring.

Porcine gamma globulin appeared to be beneficial in reducing mortality (table 1). Seventeen of the 28 deaths occurred in litters 6, 7 and 8, which received anti-hog cholera

[5] Composition of liquid simulated milk diet: Casein (or isolated soya-protein), 33.0; cerelose, 32.9; stabilized lard, 24.6; soya lecithin, 1.5; mineral mixture, 8.0% in addition to a vitamin supplement. The mineral mixture contained: KOH, 0.959; NaOH, 0.928; $CaCl_2$, 1.565; $CaCO_3$, 0.140; HCl, 0.101; MgO, 0.298; KH_2PO_4, 2.873; citric acid, 1.998; $FeSO_4$, 0.050; $CuSO_4$, 0.004; $CoCl_2$, 0.0014; $MnSo_4$, 0.0034; $ZnSO_4$, 0.0031; KI, 0.0003 and Ca $(OH)_2$, 1.00 gm. The vitamin-mixture supplied: thiamine, 0.65; riboflavin, 0.65; niacin, 2.50; choline chloride, 260.0; PABA, 2.60; folic acid, 0.052; pyridoxine, 0.65; calcium pantothenate, 1.30; vitamin E acetate, 1.0; ascorbic acid, 10.0 mg. per liter of diet, plus vitamin B_{12}, 10.0 mcg., vitamin A, 2000 I.U. and vitamin D, 200 I.U./liter of diet. The liquid diet contained 13.4% dry matter.

[6] Chlortetracycline supplied as Aureomycin by American Cyanamid Co., Pearl River, N. Y.

[7] Composition of dry diet fed from 3 to 6 weeks of age: ground yellow corn, 17.0; dried skimmilk, 40.0; soybean meal, 7.0; fish meal, 5.0; dried brewer's yeast, 1.0; trace mineralized salt, 0.5; dicalcium phosphate, 0.25; ground limestone, 0.25; stabilized lard, 2.5; sucrose, 16.5; cerelose, 10.0%, plus the following: riboflavin, 2.0; pantothenic acid, 4.0; niacin, 18.0; choline chloride, 90.0; folic acid, 0.06; vitamin B_{12}, 0.02; pyridoxine, 2.0; thiamine, 2.0; menadione, 1.0; vitamin E acetate, 10.0; chlortetracycline, 18 mg./lb. of diet. Also vitamins A and D to supply 400 and 90 I.U./lb. of diet, respectively and zinc carbonate, 100 ppm.

serum. All of these deaths occurred during the first week of life, usually following one or two days of scouring. Furthermore, all but one of the eight deaths that occurred in litters 2 and 3 occurred immediately after an injection of an antibiotic preparation given to all pigs during the first 24 hours after birth. This was probably due to an overdose of streptomycin.

It should be pointed out that all pigs born alive were used in this program. Selection of only the largest, strongest pigs from each litter would be advisable for maximum livability. It is also worth noting that the room in which the pigs were housed during the first three weeks was not free from drafts and temperature fluctuations.

The age of the survivors at market weight is given in table 2 along with that of the second generation of pigs raised in isolation and a comparable group of pigs from the main herd. It is interesting to note that the initially restricted gain of the first-generation of pigs raised in isolation (av. wt. of 5.2 lb. at 22 days of age) was offset by more rapid growth later, but not enough to equal the overall performance of pigs from the second generation raised in isolation.

An outbreak of serositis (Glasser's disease) 7 to 10 days after re-penning and immunization against hog cholera and erysipelas affected about one-half of the pigs and was fatal to one pig. Affected pigs were treated with antibiotics and recovery was complete within 2–3 days after injection.

Table 3 summarizes the incidence of AR and VPP in the main swine herd and in the first and second generations of pigs raised in isolation. Of 39 pigs slaughtered from the group raised in isolation, all have been negative for both AR and VPP as compared to an 8.8% incidence of AR and a 34.1% incidence of VPP among those being raised concurrently in the main barn. Clinical signs of AR were seen only in an occasional pig in the main barn during the first year of the eradication program despite the fact that several of the sows that had formerly raised AR-affected pigs were still in the herd. It has been observed in this herd that the incidence of AR varies widely from year to year. For example, this past year the incidence of obviously affected pigs among those being raised for market in the main herd rose sharply to approximately 20 to 30%.

Since the housing and management and the rations fed have not been identical between the swine in the main herd and those raised in isolation, and since littermate comparisons are not possible, a direct, absolute comparison of the performance of the two populations cannot be made. However, as shown in table 2 the average growth rate of first and second generation swine raised in isolation as compared to that of pigs raised on comparable rations in the main herd indicates an improvement in growth in the complete absence of AR and VPP. (Feed required per pound of gain for the three groups was 3.66, 3.60 and 3.56 lb. from weaning to market weight.) This advantage in growth rate existed despite the fact that, until the middle of the second winter, the heating system and insulation in the newly constructed isolation barn had not been installed, resulting in an extremely damp, cold environment. It is unfortunate, from the standpoint of direct comparison that littermates to the pigs raised in isolation were not raised on identical rations in the environment of the main herd, but this type of

TABLE 2. PERFORMANCE OF PIGS RAISED IN THE MAIN BARN (INFECTED) VERSUS THAT OF 1ST AND 2ND GENERATION PIGS RAISED IN ISOLATION (AR- AND VPP-FREE)

Item	Main barn	Isolation barn	
		1st generation	2nd generation
No. of pigs[a]	52	27	27
Age at 200 lb. (days)[b]	182.5	174.6	167.3

[a] Represents only pigs fed comparable rations during the entire growing-fattening period.

[b] Adjusted to 200.0 lb. by adding or deducting one day for every pound under or over 200.0 lb., respectively, at the weigh-day nearest 200 lb. Pigs were weighed at approximately two-week intervals. The unadjusted average age (days) at the weigh-day nearest 200 lb. and the corresponding average weights (lb.) as taken were, respectively, for the pigs raised in the main barn, 182.1 and 199.6; first generation pigs raised in isolation, 159.0 and 184.4; and, second generation pigs raised in isolation, 173.4 and 206.1.

TABLE 3. INCIDENCE OF AR[a] AND VPP[b] IN SWINE SNOUTS AND LUNGS EXAMINED 1958–1960

Location	No. checked (May 1958–May 1960)	Positive for AR		Positive for VPP	
		No.	%	No.	%
Main barn	91	8[c]	8.8	31	34.1
Isolation barn	39	0	0	0	0

[a] Atrophic rhinitis.

[b] Virus pig pneumonia.

[c] All eight occurred during the spring of 1960.

experimental approach was out of the realm of this project.

It should be pointed out that 36 gm. of chlortetracycline [8] per ton of feed were routinely given pigs in the main herd. Gouge *et al.* (1956) reported that 50 gm. of chlortetracycline per ton of feed stimulated gains of AR-affected pigs by as much as 15.5%.

These preliminary results provide further evidence that AR and VPP are spread only by direct contact of infected with susceptible swine. It has been established that suckling pigs are susceptible to both AR and VPP (Johnson *et al.*, 1955; Betts *et al.*, 1955; MacPherson and Shanks, 1955). Such a program provides, under reasonably practical conditions, another means of eradicating these diseases from a swine herd simultaneously. Essential steps in a successful program of this kind include:

1. Farrow sows in a clean area, preferably some distance away from other swine, and remove pigs to a location away from other swine immediately after being born. This requires strict 24-hour attendance at the time of farrowing.

2. House pigs individually in clean, draft-free, temperature-constant cages at 85° F. during the first 2 to 3 weeks after birth. The strong suckling instinct in newborn pigs encourages sucking the ears and tails of other pigs when housing as a group, causing undue commotion and unrest.

3. Provide less than *ad libitum* feed intake during the liquid-feeding period to minimize scouring.

4. Supply antibiotics in the feed and, if possible, provide a source of antibodies by injection of a commercial gamma globulin preparation.

5. Maintain strict isolation from other swine. This precludes the introduction of new breeding stock to the herd once an AR-free, VPP-free status is attained, unless such stock is known to be free of these two diseases. Artificial insemination provides the possibility of utilizing the services of an outstanding boar without the danger of introducing these diseases into the herd.

Summary

A program was undertaken to eradicate atrophic rhinitis and virus pig pneumonia from the Cornell University swine herd. This consisted of breaking the cycle of infection by removing the pigs from their dams immediately after birth and raising them in isolation from other swine. Foundation animals were selected from pigs raised in this way and were mated by natural service to boars raised with them in isolation or by artificial insemination to regular herd sires. Pigs farrowed from these matings were raised in the conventional way, the only precaution being that of maintaining strict isolation from atrophic rhinitis- virus pneumonia- infected swine.

Of 91 snouts and lungs checked from the main herd over a two-year period, 8 and 31 have been diagnosed positive for atrophic rhinitis and virus pneumonia, respectively, as compared to 0 and 0 out of 39 checked from the group raised in isolation.

These preliminary data lend further support to the evidence that these two diseases are spread only by direct contact of infected swine with susceptible swine. This provides a means of simultaneously eradicating atrophic rhinitis and virus pig pneumonia from a swine herd under reasonably practical conditions.

[8] Aurofac 2A generously supplied by American Cyanamid, Pearl River, New York.

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THE NUTRITIVE VALUE OF FALL-HARVESTED COASTAL BERMUDAGRASS HAY AS AFFECTED BY RATE OF NITROGEN FERTILIZATION AND STAGE OF MATURITY [1, 2]

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IT is difficult to make high quality grass hay in Florida during the summer months due to the high rainfall; therefore, information on the making of high quality grass hay in the fall, during a relatively dry season, can be of inestimable value to cattlemen.

In Florida, Gammon and Blue (1957) and Blue *et al.* (1957) showed that as the fertilization date was delayed after September 1, the rate of coastal bermudagrass production (i.e., yield and protein per acre) decreased markedly but that nitrogen properly applied in late August provided excellent yields of hay with a high protein content.

The object of the study reported herein was to determine the effects of two levels of nitrogen fertilization and two dates of harvesting during the Fall on the nutrient composition, digestibility and feeding value of coastal bermudagrass hay.

Experimental Procedure

Agronomic Date. Coastal bermudagrass was grown at the Suwannee Valley Station near Live Oak, Florida, on moderately-drained Klej fine sand. The plots were clipped the last week of August and 0–10–20 fertilizer was applied at the rate of 500 lb. per acre to all plots. In addition, the field was divided in half and nitrogen (ammonium nitrate) was applied at the rates of 50 and 100 lb. per acre. Each of the two plots was subdivided and one half was cut for hay in mid-October before the beginning of freezing temperatures and the remaining half was cut in early December within a week after the first killing frost. The treatments and their designations are as follows: coastal bermudagrass fertilized with 100 lb. nitrogen per acre cut for hay before frost (BF100) and after frost (AF100); coastal bermudagrass fertilized with 50 lb. nitrogen per acre cut for hay before frost (BF50) and after frost (AF50). Hay was produced in this manner during two successive years for use in winter feeding and digestion trials.

1957 Group-feeding Trial. In a completely randomized design, 20 open yearling purebred Angus and Hereford heifers were divided into four lots and fed *(ad libitum)* one of the hays plus trace mineralized salt and steamed bone meal. The duration of the feeding trial was 70 days, from December 12, 1957, to February 20, 1958. Average daily gains and feed consumption were measured. Blood data and condition scores were recorded at the beginning and end of the trial. Observations were made of reproduction performance of each animal; muscle tone and presence of follicles and corpora lutea were determined by rectal palpation at regular intervals. Each heifer was hand-mated to a previously determined fertile bull. Upon termination of the feeding trial, all the heifers were put in the same pasture and their reproduction performance was recorded during parturition and breeding the following year.

1957 Digestion Trial. A 4 × 4 Latin square conventional total collection digestion trial was conducted during the months of January, February and March. Four open yearling heifers of similar weight were fed in stanchion-type stalls and feces were collected as described by Gorski *et al.* (1957). Ten-day preliminary and seven-day collection periods were used. The feeding level was slightly above maintenance and free access was permitted to water, trace mineralized salt and steamed bone meal.

1958 Individual-feeding Trial. In a randomized block design, 16 open yearling purebred

[1] Florida Agricultural Experiment Station, Journal Series, No. 1105.

[2] Data reported were taken from a thesis presented by R. A. Alexander to the Graduate School, University of Florida, in partial fulfillment of the requirements for the Master of Science in Agriculture degree.

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Angus and Hereford heifers were divided into four lots and individually fed *(ad libitum)* one of the hay treatments plus trace mineralized salt and defluorinated phosphate. The duration of the feeding trial was 126 days, from December 9, 1958, to April 14, 1959. The same criteria were used in this trial as previously described in the 1957 group-feeding trial; however, the heifers were bred by artificial insemination.

1958 Digestion Trial. Two 4 × 4 Latin square conventional total collection digestion trials were conducted concurrently with cattle and sheep during the months of March, April and May. Four open four-year-old Hereford cows of similar weight were fed and handled by methods described for the 1957 trial. Four yearling crossbred Rambouillet-Native wethers of similar weight were fed in expanded metal collection racks. The sheep were fed in the same environment as the cows to obtain data for another project on the comparative digestibility of nutrients in forages by sheep and cattle. Ten-day preliminary and eight-day collection periods were used. The feeding level was slightly above maintenance with free access to water, trace mineralized salt and defluorinated phosphate.

Proximate analyses were made as outlined by the AOAC (1955). Nitrogen-free extract was determined empirically by difference and gross energy by bomb calorimetry with a Parr adiabatic oxygen bomb calorimeter. Statistical analyses were made as described by Snedecor (1956).

Results and Discussion

The proximate chemical composition and gross energy content of the hays fed in all experiments are combined in table 1. In the 1957 experiment, the protein content of the hay was increased more than 30% when the rate of nitrogen application was increased from 50 to 100 lb. per acre; in the 1958 experiment, it was increased only 12%.

Working with summer-cut coastal bermudagrass, Burton *et al.* (1956, 1952) and Prine and Burton (1956) have demonstrated increases of 8.0% to 19.0% in protein by increasing rates of nitrogen from 50 lb. to 900 lb. per acre. Numerous reports on other forages have indicated that nitrogen fertilization may be expected to increase the protein content and hay yield (Burton and Prine, 1958; Carey *et al.*, 1952; Cooper, 1956; Laughlin, 1953; Lewis, 1957; Ramage *et al.*, 1955, 1957, 1958; Willhite *et al.*, 1955).

No attempt is made to distinguish between the effect of frosting and the stage of maturity on hay composition and quality; although, the terms "before frost" and "after frost" were used to denote the time of harvest. With an increase in stage of maturity from October to December, the protein content of the hay from the 100-pound nitrogen treatments decreased from 8.9 to 6.7% in 1957 and from 9.7 to 8.2% in 1958, while that from the 50-pound nitrogen treatment decreased from 6.8 to 5.0% in 1957 and 8.9 to 7.1% in 1958. Increasing the stage of maturity of coastal bermudagrass by clipping at intervals of two, four, six and eight weeks after fertilization was shown by Prine and Burton (1956) and Knox *et al.* (1958) to increase the yield of hay but lowered its protein content; however, the total yield of protein per acre was affected very little.

Calcium content of the hay increased and phosphorus content decreased as the stage of maturity increased (table 2).

Cattle weight gains and voluntary feed intake are summarized in table 3. In the 1957

TABLE 1. NUTRIENT COMPOSITION OF DRY MATTER IN COASTAL BERMUDAGRASS HAY

Hay fed[a]	Protein	Crude fiber	Ether extract	Nitrogen-free extract	Ash	Gross energy
	%	%	%	%	%	therms/lb.
1 BF 100	8.93	36.31	1.11	48.35	5.30	2.08
1 AF 100	6.68	38.85	1.05	49.37	4.05	2.10
1 BF 50	6.81	35.95	1.20	50.98	5.06	2.08
1 AF 50	4.97	38.28	1.54	51.28	3.93	2.08
11 BF 100	9.65	32.72	1.33	52.50	3.80	2.05
11 AF 100	8.24	31.51	1.53	55.34	3.38	1.98
11 BF 50	8.92	30.89	1.48	54.37	4.34	1.98
11 AF 50	7.12	31.69	1.59	55.72	3.88	1.95

[a] 1957 trial designated by prefix 1; 1958 trial by 11.

TABLE 2. CALCIUM, PHOSPHORUS AND MAGNESIUM CONTENT OF COASTAL BERMUDAGRASS HAY

Hay fed	1957			1958		
	Calcium	Phosphorus	Magnesium	Calcium	Phosphorus	Magnesium
	%	%	%	%	%	%
BF 100	0.16	0.14	0.07	0.36	0.16	0.23
AF 100	0.22	0.12	0.08	0.42	0.15	0.15
BF 50	0.14	0.14	0.05	0.39	0.18	0.24
AF 50	0.18	0.13	0.08	0.45	0.13	0.17

experiment, feed intake was significantly lower ($P<0.05$) with the AF50 hay than with the other hays. This is reflected in an average daily loss in weight of 0.7 lb. which was significantly different ($P<0.01$) from weight changes on the other treatments. Feed intake and average daily gains were not significantly different between treatments BF100 and BF50; apparently, the increased rate of nitrogen fertilization did not affect appetite for hays cut at the earliest stage of maturity. Burton *et al.* (1956) compared the palatability of coastal bermudagrass hay from fields fertilized with levels of nitrogen varying from 0 to 1500 lb. per acre and found that hay intake increased with increasing rates of nitrogen.

In the lots fed AF50 hays in both 1957 and 1958, the intake of total digestible nutrients (TDN), digestible protein (DP) and digestible energy (DE) was below the National Research Council (NRC) daily nutrient requirements for wintering 700-lb. yearling cattle to gain from 0.7 to 1.0 lb. per day. These animals consistently lost weight. In the 1957 feeding trial, the daily intake of DP (0.8 lb.) and TDN (9.4) on the BF100 hay produced a 0.7-lb. per day average gain which coincides exactly with the NRC requirement. The two lots, AF100 and BF50, consumed adequate energy but their DP intakes were 0.6 and 0.5 lb., respectively; hence, their gains of 0.4 and 0.6 lb. per day were slightly below the NRC standard. In the 1958 trial, the DP, TDN, and DE requirements listed by the NRC were not completely satisfied on the BF100, AF100 and BF50 hays; hence, these deficiencies were reflected in daily gains of only 0.2 to 0.4 lb. per day. In spite of the lower gains, the apparent health, condition, growth and reproduction performance of all lots except those fed AF50 hays was satisfactory.

In the 1958 experiments, the trends in weight changes and feed intake are similar to those shown by the 1957 data although both were lower the second year. This might be the result of individual feeding. In a comparison of feeding methods, Winters and Peters (1931) reported higher gains in group-fed than individually fed steers.

The range in hemoglobin values was from 10.2 to 12.9 gm. per 100 ml. blood and hem-

TABLE 3. AVERAGE DAILY GAINS AND AVERAGE DAILY NUTRIENT INTAKE PER ANIMAL

Treatment[a]	Av. final weight	Av. daily gain	Daily intake per animal			
			Dry matter	T.D.N.	Digestible protein	Digestible energy
	lb.	lb.	lb.	lb.	lb.	therms
1 BF 100	776	0.7	15.8	9.4	0.8	17.6
1 AF 100	757	0.4	15.5	9.4	0.6	17.6
1 BF 50	767	0.6	15.1	8.8	0.5	16.5
1 AF 50	675	−0.7**	12.7*	7.1	0.2	14.1
11 BF 100	736	0.4	14.1	7.6	0.8	15.5
11 AF 100	758	0.2	14.3	8.0	0.7	15.3
11 BF 50	748	0.2	14.6	8.1	0.7	16.0
11 AF 50	673	−0.3*	11.5	6.0	0.4	11.1

[a] 1957 group-feeding trial designated by prefix 1; 1958 individual-feeding trial by 11.
* $P<0.05$.
** $P<0.01$.

TABLE 4. APPARENT DIGESTION COEFFICIENTS, DIGESTIBLE ENERGY AND TDN CONTENT OF THE COASTAL BERMUDAGRASS HAYS

Hay fed[a]	Protein	Crude fiber	Ether extract	Nitrogen-free extract	Energy	Digestible energy	Total digestible nutrients
	%	%	%	%	%	therms/lb.	%
Heifers (4)							
1 BF 100	56.0[b]	55.5	41.4	58.4	53.6	1.12	54.8
1 AF 100	55.5	57.5	40.9	57.7	53.9	1.14	55.7
1 BF 50	47.1	50.6	46.8	59.0	52.5	1.09	53.3
1 AF 50	32.2	50.7	58.6	54.1	48.9	1.02	51.1
Cows (4)							
11 BF 100	56.7	56.2	44.4	54.2	53.8	1.10	53.6
11 AF 100	54.9	56.9	52.1	57.6	54.3	1.07	56.1
11 BF 50	54.0	57.6	47.2	57.2	55.2	1.09	55.3
11 AF 50	46.8	55.1	45.5	54.3	49.3	0.98	52.7
Sheep (4)							
11 BF 100	63.4	60.9	52.3	58.7	60.4	1.24	58.4
11 AF 100	57.3	59.9	58.3	60.4	57.9	1.14	59.0
11 BF 50	58.1	58.7	59.7	60.0	57.4	1.14	57.9
11 AF 50	48.5	53.9	55.8	52.5	50.5	0.99	51.8

[a] 1957 trial designated by prefix 1; 1958 trial by 11.
[b] Each figure represents an average of four animals.

atocrit values were 41.5 to 50.3%; however, no consistent differences were detected between treatments. The condition scores ranged from 5.0 (utility) to 6.2 (standard) with no significant differences between treatments.

A summary of the digestibility data is presented in table 4. The apparent digestibility of protein is related to the protein content in the forage on a within-year basis. However, these data indicate that there are factors other than level of protein influencing protein digestibility because a difference was observed between years, e.g., the average protein content was higher in the 1958 than in the 1957 hays but the protein digestion coefficients were not increased proportionately. With the high rate of nitrogen (100 lb. per acre), protein digestibility was decreased only slightly by an increased stage of maturity; whereas, at the lower rate of nitrogen (50 lb. per acre), protein digestibility decreased from 47.1 to 32.2% with heifers; 54.0 to 46.8% with cows and 58.1 to 45.5% with sheep.

Digestibility studies were employed by Knox *et al.* (1958) to ascertain the effect of one rate of nitrogen fertilization and varying stages of maturity from two to eight weeks upon the nutrient availability of coastal bermudagrass. The apparent digestibility of protein and total digestible nutrients decreased with increasing stages of maturity. The greatest decline in digestibility occurred between six and eight weeks in the stage of maturity.

Dry matter and energy digestibility fol-

TABLE 5. ANALYSIS OF VARIANCE AND TREATMENT COMPARISON OF APPARENT DIGESTION COEFFICIENTS AND TDN

Source of variation	df	Mean squares					
		Dry matter	Protein	Crude fiber	Nitrogen-free extract	Energy	TDN
Total	31						
Treatment	(3)	44.39*	318.14**	29.78*	53.03*	89.62**	43.81*
50 vs. 100 lb. N	1	36.34	308.14**	37.41	23.63	96.61**	46.28*
BF 50 vs. AF 50	1	91.20**	283.08**	51.84*	109.20**	168.35**	75.69**
BF 100 vs. AF 100	1	5.64	63.21*	0.09	26.27	3.90	9.45
Species	1	33.83	112.88**	28.88	34.65	93.16**	44.55*
Treatment x species	3	12.53	10.13	12.79	1.45	11.18	8.29
Periods	6	39.34**	32.53	59.84**	34.97*	28.24*	36.22*
Animals	6	16.79	16.57	18.69	16.59	25.13*	15.38
Error	12	7.98	10.91	8.55	10.39	6.78	7.87

* $P<0.05$.
** $P<0.01$.

lowed trends similar to the protein digestibility. The TDN values increased slightly as the nitrogen level was increased from 50 to 100 lb. per acre. Yet animal gains were markedly different apparently due to differences in dry matter intake protein content and digestibility. The weaknesses of the TDN system in the evaluation of forages have been reviewed by Swift (1957). According to Crampton *et al.* (1957), voluntary forage intake and energy digestibility are the best criteria for estimating the nutritive value of forages fed as the entire energy part of the ration.

Digestibility data by Poulton *et al.* (1957) with orchardgrass, Hopkins *et al.* (1959) with mountain meadow grass, and Markley *et al.* (1959) with bromegrass indicate that the level of nitrogen fertilization influences the digestibility of nutrients in grasses.

Analysis of variance and treatment comparisons of the apparent digestion coefficients and TDN are presented in table 5. The most significant effect was due to the increased stage of maturity at the lower nitrogen level. Nitrogen level had a highly significant ($P<0.01$) effect on protein and energy digestibility.

Summary

Digestibility and feeding trials were conducted with cattle and sheep to study the effects of two rates of nitrogen fertilization, 50 and 100 lb. per acre, and two dates of harvest during the Fall, mid-October and early December, on the nutrient composition, digestibility and feeding value of coastal bermudagrass hay.

Hay consumption decreased and weight losses occurred with the late-cut, low-nitrogen treatment hay while the other hays when fed free choice with a mineral supplement were adequate for wintering open yearling replacement heifers. There were no significant differences between treatments in frequency of estrus, ovulation rate, conception rate, blood data and condition scores.

The content and digestibility of protein of the hay varied directly with rate of nitrogen application, on a within-year basis; they were significantly lower ($P<0.01$) in the late-cut, low-nitrogen hay. The protein content of the hay was increased up to 30% by increasing the rate of nitrogen fertilization from 50 to 100 lb. per acre and was decreased by harvesting in December at a late stage of maturity and after a killing frost.

These data demonstrate a method of making high quality grass hay in the high rainfall area of the Gulf Coast States. Coastal bermudagrass to be cut for hay or reserved for winter grazing in the Gulf Coast States should receive at least 50 lb. of nitrogen per acre if the forage is to be used within 6 to 8 weeks after the nitrogen application. If the stage of maturity at time of harvesting or grazing exceeds 8 weeks, at least 100 lb. of nitrogen per acre should be applied.

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VARIATIONS IN SOME BIOCHEMICAL AND HISTOLOGICAL CHARACTERISTICS OF BOVINE CORPORA LUTEA DURING EARLY PREGNANCY [1]

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THE progestogen concentration in corpora lutea of cows was found to be lower at the 56th day of pregnancy (Zimbelman *et al.*, 1961) than at the 14th day of the estrual cycle (Loy *et al.*, 1960). Histological examination of the corpora lutea in these two studies gave evidence that the difference was probably a reflection of a difference in the proportion of functional luteal cells. A low level of function in the corpus luteum of pregnancy in the cow might not be unexpected at later stages of gestation in view of the findings of McDonald *et al.* (1953) that pregnancy can be maintained in the absence of this gland; it was unexpected, however, that a decline in function would begin at so early a stage.

This investigation was made up of two experiments intended to determine the time that the change in the level of function of the corpus luteum occurs. Comparisons were made between the 14th day of estrual cycle and selected stages during early pregnancy.

Materials and Methods

All animals used in the two experiments were virgin heifers of varying age and weight; they were more alike in age within experiments than between the two experiments. All times were expressed as elapsed-days from the time the heifer was first found in heat. Only those bred heifers with normal-appearing embryos at the time of slaughter were included as pregnant heifers in the analysis of the results.

In experiment one, groups of heifers were studied at 14, 28 and 42 days of pregnancy; a group of unbred heifers at the 14th day of the estrual cycle was also studied. The latter group will also be referred to as the day-14 cyclic group. When the results of experiment one showed that changes were already apparent by day-28, experiment two was designed so as to study the interval immediately following day-14. Groups of heifers were studied at 18, 23, and 28 days of pregnancy. The major question considered was whether the change was uniform throughout this interval or whether more rapid changes in the corpus luteum occurred during some shorter span of time.

Each corpus luteum was weighed fresh and a small portion was removed and fixed in Bouin's solution for histological examination. The remainder of the gland was stored frozen in 95% ethanol and chemical determinations for progestogens, sterols, and nucleic acids were made in duplicate at a later date.

The basic method for progestogen analysis was that of Loy *et al.* (1957), with the further modifications described by Loy *et al.* (1960). The latter report pointed out the presence of a contaminating steroid, thought to be 4-pregnene-20β-ol-3-one. The behavior of this substance in the paper chromatography system indicated it to be a more polar steroid than progesterone. Consequently, it was postulated that significant amounts of this compound might be present in separatory funnels 8 and 9 of the countercurrent distribution system. Two fractions were then saved from the countercurrent distribution system, the usual fraction, which was the contents of the center five funnels (3 through 7), plus an additional fraction made up of the contents of funnels 8 and 9. These two fractions were run separately on paper chromatography; the entire procedure will be called method A and was used in experiment one. Since significant amounts of 4-pregnene-20β-01-3-one were found to be present in separatory funnels 8 and 9, the data used in experiment one were the totals of that contained in both fractions (funnels 3–7 and 8–9). All values were based on the quantitative estimation following paper chromatography; this was calculated using the formulas of Reineke (1956) following deter-

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mination of the optical densities at 224 and 240 mu.

The progestogen analyses for experiment two mere made by method B which substituted a simple solvent partition for the countercurrent distribution system. The paper chromatographic partition in a Bush type system of Skelly-solve B and 80% methanol (Reineke, 1956) was used routinely in this method. The entire procedure is discussed in more detail elsewhere (Zimbelman, 1960).[3] When paper chromatography was used routinely in the progestogen analysis, a mixture of crystalline 4-pregnene-20β-ol-3-one[4] and progesterone was run on a strip adjacent to strips containing luteal extracts. The behavior of the two substances in the luteal extracts was the same as that of the crystalline substances. An infrared spectrum of the slower-moving component was identical to that of crystalline 4-pregnene-20β-o1-3-one.

Since Zander *et al.* (1958) demonstrated that 4-pregnene-20β-o1-3-one has progestational activity by two biological tests, it seemed important to estimate the amount of 4-pregnene-20β-o1-3-one separately from that of progesterone. This made the paper chromatography step a necessary one in the total procedure. Total progestogen will be used to denote the sum of progesterone plus 4-pregnene-20β-o1-3-one.

The method of sterol analysis involved precipitation with digitonin and color development with Liebermann-Burchard reagent (Sperry and Webb, 1950). There appeared to be at least two components. One component gave maximum color development between 30 and 40 minutes and is presumed to be largely cholesterol. There was a small amount of "fast-acting" sterol which produced maximum color at 90 seconds (Miller and Baumann, 1954), the nature of this component(s) is not known. The formulas used to calculate the amount of sterol per sample are given elsewhere (Zimbelman, 1960).

For nucleic acid analysis the tissue was processed in essentially the manner described by Schneider (1957) with the exception that the lipid extraction step preceded the cold trichloroacetic acid (TCA) wash for removal of acid soluble material. Extraction was performed with hot (90° C.) TCA for 20, 20 and 60 minutes. The nucleic acids were estimated colorimetrically by the diphenylamine reaction for deoxyribonucleic acid (DNA) and the orcinol reaction for ribonucleic acid (RNA); the latter was corrected for DNA content. The values are expressed in absolute units (mg.), but are based on the amount of color given by known amounts of nucleic acids of origin other than bovine. Thus, the values are relative to the standards used (obtained from California Biochemical Corporation).

The concentration of a substance will be referred to in terms of mcg. or mg. per gram of fresh luteal tissue; the content will refer to the mcg. or mg. per gland (concentration times total fresh weight).

Results

Progestogen Content. Progesterone concentration and content in experiment one were lower ($P<0.01$) at day-28 and -42 of pregnancy than at day-14 (table 1). There was no significant difference between the day-14 groups, one being pregnant and the other unbred. The mean values for 4-pregnene-20β-o1-3-one were not different for the three pregnant groups. The means were greater for the 28- and 42-day groups than for the cyclic group, but this comparison is not valid.[5] Total progestogen concentration and content were significantly lower in the groups at day-28 and -42 than in the day-14 pregnant group; the two day-14 groups did not differ from each other (table 1).

The progesterone levels in this study are in general agreement with those reported by Gorski *et al.* (1958) and Kristoffersen (1960), but are considerably higher than those reported by Melampy *et al.* (1959). The levels of 4-pregnene-20β-o1-3-one tend to be somewhat higher than those of Gorski *et al.* (1958); Kristoffersen (1960) reported the presence of this substance but did not measure it in all cases.

It appeared that the greatest decrease in the progestogen content in experiment one occurred between day-14 and -28, so experiment two was designed to study further the interval between day-14 and -28. A regression analysis

[3] The authors are indebted to W. H. McShan, Department of Zoology, for advice in chemical methodology.

[4] Crystalline 4-pregnene-20β-ol-3-one and the infrared analysis were supplied by Alan F. Krivis of The Upjohn Company, Kalamazoo, Michigan.

[5] Due to an oversight, only separatory funnels 3 through 7 were saved for the cyclic group, whereas other groups are based on funnels 3 through 9. This would lead to an underestimation of progesterone by about 3%, which is negligible; but 4-pregnene-20β-ol-3-one would be underestimated by a greater and unknown amount. Comparison of progesterone values of the cyclic group and the 28- and 42-day pregnant groups should not be invalidated, however, since the latter groups are significantly lower despite the underestimation of the day-14 cyclic value.

TABLE 1. DIFFERENCES IN THE CORPUS LUTEUM AT VARIOUS REPRODUCTIVE STAGES IN EXPERIMENT ONE

Comparison	Day-14 estrual cycle	Day-14 pregnancy	Day-28 pregnancy	Day-42 pregnancy	F value[a]
Number of animals	6[b]	5	9[c]	5	
Progestogen conc.[d]					
20β-ol[e]	2.5	5.9	8.0	9.1	4.47*
Progesterone	31.4	35.2	18.0	9.9	17.83**
Total	33.9	41.1	26.0	19.0	10.54**
Progestogen content[f]					
20β-ol	13.8	33.8	46.2	51.2	3.38*
Progesterone	178.4	198.2	101.5	52.0	18.24**
Total	192.2	232.0	147.7	103.2	9.01**
Cholesterol conc.[d]					
Free	1950	2260	2729	2414	10.20**
Esterified	260	265	193	147	2.41
Total	2210	2525	2922	2561	6.73**
Fast acting sterol conc.[d]					
Free	130	127	110	132	0.77
Esterified	28	52	32	31	1.52
Total	158	179	142	163	0.93
Nucleic acid conc.[g]					
DNA	3.43	3.82	3.73	3.38	1.71
RNA	5.43	5.27	5.04	4.46	4.28*

[a] From analysis of variance of difference among the four stages.
[b] One value missing for nucleic acid concentration due to accident.
[c] Three values missing for progestogen determinations due to accident.
[d] Mcg. per gram fresh luteal tissue.
[e] 4-pregnene-20β-ol-3-one.
[f] Mcg. per gland.
[g] Mg. per gram fresh luteal tissue.
* $P<0.05$.
** $P<0.01$.

was employed to determine whether the decrease was continuous throughout this interval or was occurring more during a part of the interval.

The regression of total progestogen content per day of pregnancy between day-14 and -28 of experiment one was —6.03 mcg. per day; for experiment two the regression from day-18 to -28 was —6.32 mcg. per day. The difference between these two regressions was not statistically significant (table 2), so the within-experiment sums of squares and cross-products were pooled. The pooled regression coefficient was —6.12 mcg. per day ($P<0.01$). The pooled regression coefficient was checked for deviation from linearity, but no significant deviation was found (table 2). Comparable regressions for progesterone content were: experiment one, —6.91; experiment two, —4.27; pooled —6.07 mcg. per day. None of the regressions of 4-pregnene-20β-ol-3-one was significant, an observation consistent with the

TABLE 2. REGRESSION OF STEROID LEVELS ON DAY OF PREGNANCY

Comparison	Exp. I[a]	Exp. II[b]	F value for difference between experiments	Experiments I and II pooled[c]	F value for deviation of pooled regression from linearity
Progestogen content					
20β-ol[d]	0.88	—2.04	2.64	—0.05	....
Progesterone	—6.91**	—4.27	1.01	—6.07**	1.05
Total	—6.03**	—6.32	0.01	—6.12**	0.95
Free cholesterol conc.	33.5*	41.1*	0.11	35.6**	0.95

[a] Day-14 and -28 of pregnancy.
[b] Day-18, -23, and -28 of pregnancy.
[c] Within-experiment sums of squares and cross-products pooled.
[d] 4-pregnene-20β-ol-3-one.
* $P<0.05$.
** $P<0.01$.

previous analyses which showed no significant difference between groups in the mean content of this substance.

Thus, a gradual but significant decrease in progesterone content occurred between day-14 and -28 of pregnancy. In contrast, 4-pregnene-20β-o1-3-one did not change significantly.

Sterol Content. Due to the studies which indicate cholesterol to be a precursor of steroid hormones (Everett, 1947; Samuels, 1958), cholesterol levels were measured to determine their relationship to the changing progesterone levels.

The means for free and for esterified fast-acting sterol did not vary significantly (table 1). Free cholesterol concentration at day-28 in experiment one, however, was higher ($P < 0.01$) than that at day-14 of pregnancy. The concentration at day-42 was intermediate and not significantly different from either day-14 or -28. The mean values for esterified cholesterol were not significantly different, but the tendency was in the direction of a decrease with time. Thus, the only definite variation with stage of pregnancy was in free cholesterol concentration.

Again in experiment two only free cholesterol appeared to vary with stage of pregnancy. Analysis of covariance revealed that a significant positive regression of free cholesterol on day of pregnancy occurred up to day-28 in both experiments. The pooled regression coeffiicent (35.6 mcg. per gram per day) was significant ($P<0.01$), and could not be demonstrated to deviate from linearity (table 2).

Thus, during the initial period of the decrease in progestogen content a significant increase in cholesterol occurred. This rise appeared to be followed sometime later by a decrease, so that the level at day-42 of pregnancy was more like the level at day-14 than at day-28. Boyd and Elden (1935) also found that the concentration of free cholesterol varied inversely to that of progestogen; the latter determinations were based on a bioassay utilizing the progestational proliferation of the rabbit uterus as the end-point.

Davis *et al.* (1956) suggested that the cholesterol available in the corpus luteum has a two fold origin: (1) mobilization from the blood plasma, and (2) local synthesis within the gland. The increase in free cholesterol observed in this study may be interpreted in general terms, as follows: As the use of cholesterol in the synthesis of progestogens decreased, there was an increase in cholesterol. However, the negative feedback mechanisms then caused a decrease in cholesterol synthesis, and a subsequent decrease in cholesterol content was observed. A lag in time between the decrease in progestogen synthesis and the rise and subsequent fall of the cholesterol level may result because there are several intermediate steps between cholesterol and progesterone.

Nucleic Acid Content. Interpretation of DNA values was made on the assumption that the amount of DNA is constant per cell for normal cells of a given organism (Vendrely and Vendrely, 1956). Thus, changes in DNA are assumed to be a reflection of changes in number of cells. DNA determinations were made for experiment one only, the differences between groups were not significant (table 1).

Potter (1957) discusses the relationships between DNA, RNA, enzymes and protein synthesis. Under his concept the primary factor in determining enzyme amount is the so-called enzyme forming system, which is equated with a specific RNA-protein. In the present study, RNA may then be considered as a measure of enzyme forming systems some of whose formed enzymes are used in the synthesis of progestogens by the luteal cells. If the decreased progestogen content were simply a function of increased release of steroid from the gland, enzyme requirements should remain high. A decrease in RNA might be indicative of lesser progestogen synthesis.

In the present study, RNA concentration appeared to undergo a general decline with time. The 42-day level was lower ($P<0.05$) than that of the other three groups, which were not significantly different from each other (table 1). These results support the idea of lowered cellular activity with increasing length of pregnancy, and would suggest decreased progestogen synthesis as a reason for lowered progestogen content of the corpus luteum. RNA determinations were not made in experiment two, as experiment one indicated that the difference was not readily apparent until after day-28.

Corpus Luteum Weight. Mean corpus luteum weights did not differ significantly in experiment one (table 3). Hammond (1927) concluded that there was no marked increase in size of the corpus luteum of the cow during pregnancy. Melampy and Gay (1958) found no correlation between corpus luteum weight and stage of pregnancy in slaughterhouse cows. Foley and Reece (1953) stated that the corpus luteum showed a slight, though con-

stant, increase in average weight between 25 and 45 days of pregnancy.

In experiment two the mean corpus luteum weight tended to be lower than in experiment one. The mean weights of the 18- and 23-day pregnant groups were similar to those of experiment one (table 3); however, the mean weight of the corpora lutea of the 28-day group was considerably lower than that of any other group. If this were a real difference, it would require that the corpora lutea decrease in size between day-23 and -28. Because these corpora lutea were examined frequently by rectal palpation from a few days after breeding until slaughter and no decrease in size seemed to occur, this difference is believed to be due to chance rather than to stage of pregnancy.

TABLE 3. WEIGHT OF CORPUS LUTEUM AND PERCENTAGE OF DIFFERENT TYPE CELLS AT VARIOUS REPRODUCTIVE STAGES

Comparison	Stages studied in experiment one				
	Day-14 estrual cycle	Day-14 pregnancy	Day-28 pregnancy	Day-42 pregnancy	F value[a]
Number of animals	6	5	9	5	
Corpus luteum weight (gm.)	5.78	5.64	5.26	5.48	0.39
Percentage of cells					
Types I and II	77.7	50.2	13.0	12.6	36.07**
Type III	11.0	30.5	54.9	56.4	47.58**
Types IV and V	10.9	18.7	30.1	30.5	27.51**

	Stages studied in experiment two			
	Day-18 pregnancy	Day-23 pregnancy	Day-28 pregnancy	
Number of animals	5	5	5	
Corpus luteum weight (gm.)	5.14	5.24	3.99[b]	5.35*
Percentage of cells				
Types I and II	44.8	14.5	12.1	9.10**
Type III	33.2	51.1	57.0	11.17**
Types IV and V	21.6	32.3	29.5	2.37

[a] From analysis of variance of differences among all the stages in the experiment.
[b] Palpation of the ovaries during the interval from day-23 to -28 in these animals did not reveal a decrease in size.
* $P<0.05$.
** $P<0.01$.

Histology. After fixation in Bouin's solution and paraffin embedding, the tissue was sectioned at 10 microns. Sections were stained in hematoxylin and eosin for classification of the cell types according to Foley and Greenstein (1958). The percentages of total cells classified were calculated for the different categories and these were transformed to angles for statistical analysis.

The percentages for the different categories of cells were very nearly the same for the 23-day group, the two 28-day groups, and the 42-day group (table 3). Day-14 and -18 of pregnancy were also very much alike in all three categories, and had more ($P<0.01$) functional (type I and II) cells and fewer ($P<0.01$) type III cells than all later stages of pregnancy. The corpora lutea from the heifers on day-14 of the estrual cycle had a higher percentage of functional cells than all other groups.

Changes in histology seem reasonable since there was a change in progestogen content; a somewhat more gradual change, such as was found in steroid content, might have been expected however. The differences in histology between day-18 and later stages were fairly abrupt. There was also a greater percentage of functional cells in the 14-day cyclic group than in the 14-day pregnant group, even though there was no difference in progestogen content. Although this difference was under hypothetical test, confirmation is desirable because of the potential importance of a morphological indicator at this early stage that the corpus luteum has learned that the uterus contains an embryo.

Stimulation of the uterine cervix, by means

of a plastic insemination tube, during estrus was done in a collateral study but had no demonstrable effect on either the progestogen content or the histology of the corpus luteum at day-14 of the estrual cycle (Zimbelman, 1960). Thus any differences at day-14 would be presumed to be due to the presence of the embryo rather than to being conditioned by cervical stimulation associated with breeding. The embryo at 14 days is so small, however, that any action it may have is presumed to result from aspects other than its mass.

Foley and Greenstein (1958) reported that type III cells were more common between 17 and 22 days of pregnancy than at 16 days. This observation is in agreement with the present study. They later suggest that there is an increase in type II cells with advancing gestation, which is in contrast to the present study. The present study tends to agree with the assumption that the type I and II cells are the functional cells, at least there is a general positive relationship between these cells and the progestogen content.

General Discussion

It is concluded that the corpus luteum of pregnancy is functioning at a lower level at day-42, for example, than at day-14. Decrease in RNA concentration and percentage of functional cells are in accord with an interpretation of decreased progestogen synthesis. The initial increase in cholesterol may be interpreted as an accumulation due to decreased utilization in progestogen synthesis. All three of these characteristics are individually subject to an alternative interpretation; however, a greater number of assumptions would have to be made to offer an alternative explanation for all of these observed results. If the increased cholesterol were interpreted as providing more cholesterol for progestogen synthesis, it should remain at a higher level rather than decrease after the initial increase.

The decreased progestogen content might be expected if progestogen synthesis were occurring at a lower rate, and would be necessary if the corpus luteum has no mechanism whereby it can store active progestogens. If the corpus luteum has a mechanism whereby it can store active progestogens, other alternative interpretations might be offered.

The corpus luteum is necessary for the maintenance of pregnancy in the cow until about the 200th day of pregnancy (McDonald *et al.*, 1953), but demands on the corpus luteum may not be so great as pregnancy advances. The fact that the 4-pregnene-20β-o1-3-one content does not decrease or perhaps even increases slightly may be an important factor in interpreting this apparent decrease in luteal function. The fact that this compound has progestational activity (Zander *et al.*, 1958) makes it a reasonable suggestion. However, we need to have information on the activity of this substance in the maintenance of pregnancy in the cow.

Summary

Two experiments involving 40 heifers were used to study the level of function of the corpus luteum during early pregnancy. Experiment one was a comparison of day-14 of the estrual cycle and day-14, -28, and -42 of pregnancy. Experiment two compared day-18, -23, and -28 of pregnancy.

Progesterone and total progestogen were lower ($P<0.01$) on day-28 and -42 of pregnancy than on day-14. A significant ($P<0.01$) negative regression of progesterone and total progestogen content on day of pregnancy up to day-28 was found (—6.07 and —6.32 mcg., respectively). These regressions appeared to be linear. The level of 4-pregnene-20β-o1-3-one did not appear to vary with stage of pregnancy. Free cholesterol concentration increased significantly between day-14 and -28, but tended to return toward the day-14 level by day-42. The regression of free cholesterol between day-14 and -28 of pregnancy was 35.6 mcg. per gram per day ($P<0.01$) and appeared to be linear. Mean concentrations of DNA were not different, but RNA decreased with increasing length of gestation. A decreased percentage of functional cells was apparent histologically at day-23 of pregnancy and at later stages as compared to day-14 or -18 of pregnancy. The latter named stages in turn had fewer functional cells than the unbred heifers on day-14 of the estrual cycle.

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EFFECT OF EXOGENOUS PROGESTERONE ON THE BOVINE CORPUS LUTEUM OF EARLY PREGNANCY [1]

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AN injection of progesterone caused partial suppression of the growth of corpora lutea in unbred heifers when made on day-1 of the estrual cycle but not when made on day-5 (Loy *et al.*, 1960). However, a reduction in progesterone content and in percentage of functional cells occurred as a result of injections begun at either time. Exogenous progesterone gave partial inhibition of the growth of the corpus luteum of the bred ewe also, but no evidence of regression was obtained from study either of the histological structure or of the weights at different stages (Zimbelman *et al.*, 1959). Progesterone injections, on the other hand, caused marked regression of corpora lutea in pregnant and in hysterectomized gilts (Sammelwitz *et al.*, 1956; Spies *et al.*, 1959, 1960), but did not appear to have an effect on corpora lutea of the estrual cycle (Sammelwitz and Nalbandov, 1958; Aldred *et al.*, 1959). The present study was made to determine the action of progesterone on the corpus luteum of the heifer during early pregnancy for comparison to that found in the unbred heifer, the bred ewe, and the pregnant sow.

Materials and Methods

Progesterone given in a starch suspension was injected into nine heifers on day-35, -42, and -49 of pregnancy in a dosage of 1 mg. per pound body weight. These heifers, together with 10 untreated heifers which served as controls, were slaughtered at day-56 of pregnancy. Embryos were removed from the uterus and checked for normality on the basis of crown-rump length and general gross appearance.

Corpora lutea were removed from the ovaries and weighed on a milligram balance. A small portion was removed and fixed in Bouin's solution for histological study. Chemical analyses for progestogens were made in the same manner as described by Loy *et al.* (1960). Upon subsequent analysis by paper chromatography of the pooled material from both the control and treated groups, it was found that approximately 15 to 20% of the ultraviolet absorbing material was due to a compound other than progesterone. This other compound is believed to be 4-pregnene-20β-o1-3-one for reasons presented by Loy *et al.* (1960) and Zimbelman *et al.* (1961a).

Progestational activity of 4-pregnene-20β-o1-3-one in the rabbit and the mouse was demonstrated by Zander *et al.* (1958). Inasmuch as this compound must also be considered as a progestogen, total progestogen will be used in reference to the sum of progesterone and 4-pregnene-20β-o1-3-one, although the biological activity of the latter compound in the cow is unknown.

Results and Discussion

The crown-rump lengths of embryos from the control group ranged from 52 to 64 mm. with a mean of 58.0 mm. and from the progesterone-treated group, 49 to 65 mm. with a mean of 58.1 mm. All embryos were considered to be normal. The average corpus luteum weight from the control heifers was 5.41 gm. as compared to 4.85 gm. from the progesterone-treated heifers (table 1). Since this difference is not statistically significant, the exogenous progesterone did not appear to cause any measurable regression of established corpora lutea which is similar to the results in unbred heifers when the injections were begun on day-5 of the estrual cycle (Loy *et al.*, 1960) and in the bred ewe (Zimbelman *et al.*, 1959). On the other hand, these results differ from those in pregnant gilts (Sammelwitz *et al.*, 1956; Spies *et al.*, 1959, 1960).

The mean progestogen concentration in the treated group was 7.7 mcg. per gm. of fresh

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[2] Agent of the Dairy Cattle Research Branch, U.S.D.A.

TABLE 1. EFFECT OF EXOGENOUS PROGESTERONE ON THE CORPUS LUTEUM AT DAY-56 OF PREGNANCY

Comparison	Control	Treated	Probability[a]
Number of animals	10	9	....
Corpus luteum weight (gm.)	5.41	4.85	>0.25
Progestogen concentration[b]	15.3	7.7	0.06
Progestogen content[c]	83.5	35.7	<0.05

[a] Probability of difference between control and treated.
[b] Mcg. per gram fresh luteal tissue.
[c] Mcg. per gland.

tissue as compared to 15.3 mcg. per gm. in the control group. The progestogen content (total amount per gland) was 35.7 mcg. in the treateds compared to 83.5 mcg. in the controls; this difference was statistically significant ($P<0.05$). Information on the progestogen content of corpora lutea from progesterone treated and control ewes is not known, but a decreased progestogen content was caused by progesterone injections into unbred heifers during the estrual cycle (Loy *et al.*, 1960). Progesterone injections into pregnant and hysterectomized gilts appeared to cause lowered function of corpora lutea; treated groups always had a lower mean progesterone concentration although it was not statistically significant (Spies *et al.*, 1959, 1960).

Histological examination of sections stained with hematoxylin and eosin was planned to compare the percentage of functional cells in a manner similar to that used in the studies of the bred ewe and unbred heifer. The corpora lutea of both the control and treated groups at day-56 of pregnancy contained so few functional cells (types I and II of Foley and Greenstein, 1958) that comparison was not possible. The general picture was mostly of cell types III and IV with only an occasional type II cell. A variable degree of occurrence of vacuoles of different sizes appeared in luteal cells of both groups. In addition there appeared to be somewhat more connective tissue than one finds on day-14 of the estrual cycle, as well as more apparent shrinkage of luteal cells. McNutt (1927) found that throughout pregnancy there was a gradual histological retrogression with complete destruction of certain luteal cells. Although he stated that this was not as marked during the first half of pregnancy, the histological picture of the present study indicates that it is obvious by 2 months of pregnancy if compared to corpora lutea from unbred animals on day-14 of the estrual cycle.

It seems reasonable to assume that the effect of progesterone on the corpus luteum is mediated by the pituitary gland. No definite evidence is available to indicate whether the pituitary control over the bovine corpus luteum is mediated by the known pituitary gonadotrophins (FSH and LH), by pituitary lactogenic hormone, by an unknown luteotrophic hormone, or by some particular combination of the known hormones. Injections of pituitary lactogenic hormone did not prolong the life of bovine corpora lutea (Smith *et al.*, 1957).

It is generally accepted that LH provides the stimulation for ovulation and formation of the corpus luteum, but its role after the corpus luteum is formed is unclear. Progesterone inhibited corpus luteum formation in the rat, but did not appear to affect established corpora lutea of either pregnancy or pseudo-pregnancy (Astwood and Fevold, 1939). This suggested that some hormone other than LH was responsible for the continued function of formed corpora lutea and later it was shown that lactogenic hormone was luteotrophic in the rat (Astwood, 1941). The inhibition of ovulation but not of follicular development by progesterone injections has indicated that progesterone interfers with the release of LH from the pituitary with little effect, if any, on FSH secretion in the rat (Astwood and Fevold, 1939), ewe (Dutt and Casida, 1948), cow (Ulberg *et al.*, 1951), and sow (Foote *et al.*, 1958).

Everett (1947) suggested that the ratio of LH/LTH determines the functional level of the rat corpus luteum. The formed rat corpus luteum can persist in the absence of LH (Greep, 1938), but probably does not function in the absence of LTH (Astwood, 1941). With a given level of LTH the functional activity may be increased by small doses of LH even though large doses of LH appear to be luteolytic.

Without any modification of the rat hypothesis expressed above, the results of this study might be explained as due to progesterone inhibiting pituitary release of: (1) LH, or (2) LTH, or (3) LH and LTH. The action of lactogenic hormone as LTH in the cow has not been established. If the hypothesis eventually has to be modified to omit LTH, an optimal level of LH might still be associated with maximal corpus luteum function. The

hypothesis could still allow for high levels of LH to be luteolytic, and levels lower than optimum would result in a lower level of function by the corpus luteum. In line with the modified hypothesis the results of this study would be interpreted that progesterone decreased LH release which led to lowered progestogen content of the corpora lutea.

Extensive studies with LH, and FSH also, should be carried out to aid in our understanding of the basic mechanism of control of the corpus luteum in farm animals. If neither LH nor FSH alone nor both in combination are luteotrophic, this would encourage a search for a now unknown luteotrophic hormone. An initial attempt in studies of pituitary gonadotrophins has been made (Zimbelman *et al.*, 1961b).

Summary

The effects of injecting progesterone in starch suspension on day-35, -42, and -49 of pregnancy were studied in nine heifers. Ten uninjected heifers served as controls, animals from both groups being slaughtered on day-56 of pregnancy. Progesterone had no significant effect on corpus luteum weight; the mean weight for treateds was 4.85 gm. as compared to 5.41 gm. for controls. A significant ($P < 0.05$) decrease in the progestogen content from 84 mcg. per gland in the controls to 36 mcg. in the treated group resulted from injections of progesterone. Histological examination of the corpora lutea from untreated heifers indicated a marked difference from the histological picture present at day-14 of the estrual cycle in other studies.

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THE EFFECT OF FEEDING PELLETED HAY, PELLETED EAR CORN AND A TRANQUILIZER TO FATTENING CATTLE [1, 2, 3]

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INCREASES in daily gain, feed intake and feed efficiency as a result of feeding pelleted high roughage or all roughage rations to beef cattle have been reported (Webb and Cmarik, 1958b; Wallace *et al.*, 1959; Weir *et al.*, 1959). However, the feeding of a pelleted complete fattening or high energy ration has often resulted in little or no increase in weight gain and a decrease in daily feed intake (Perry *et al.*, 1958; Burroughs *et al.*, 1959b; Weir *et al.*, 1959). Data are limited on the feed lot performance of cattle fed a small amount of pelleted or baled hay in combination with pelleted or ground ear corn.

Recently, results of several experiments have indicated that feeding the tranquilizer hydroxyzine (Tran-Q) at low levels has enhanced the feed-lot performance of fattening cattle (Ralston and Dyer, 1959; Sherman *et al.*, 1959). Others have not obtained such results (Burroughs *et al.*, 1959a; Perry *et al.*, 1960) which suggests a need for additional research.

The purpose of this study was to investigate the feed lot performance and certain carcass characteristics of beef cattle when fed: (1) a limited amount of pelleted or baled hay; (2) pelleted or ground ear corn; (3) a tranquilizer.

Experimental Procedure

Trial I. Sixty-four good-to-choice yearling Hereford steers were allotted on a weight and feeder grade basis into eight lots of eight each. The lots were randomized, two per treatment, into four treatments: (1) baled hay-ground ear corn; (2) pelleted hay-ground ear corn; (3) baled hay-pelleted ear corn; (4) pelleted hay-pelleted ear corn. Hydroxyzine, 2.5 mg. per head daily, was fed to one lot of steers in each treatment group; the other lot of each group served as the tranquilizer control. Ear corn (either ground or pelleted) was fed to appetite and alfalfa-brome hay was fed at the rate of 4 lb. per head daily. Three-sixteenth inch pellets were made by grinding hay and ear corn through a 3/16-inch screen previous to pelleting. Linseed oil meal was fed at the rate of 1.5 lb. per head daily. Corn silage was fed at the rate of 15 lb. per head daily for the first 88 days on trial, but it was not fed the last 56 days. Diethylstilbestrol and oxytetracycline (Terramycin) were mixed into the protein supplement to supply 10 mg. and 80 mg. per head daily, respectively. Water, trace mineralized salt and a mineral mixture (2 parts steamed bone meal to 1 part stock salt) were available free-choice. The cattle were hand-fed twice daily. Individual initial and final weights were taken after the cattle had been off feed and water for approximately 16 hours (overnight). Carcass data were obtained from ribbed carcasses after a 48-hour cooler shrink.

Trial II. Sixty-four good-to-choice grade yearling Hereford heifers were used in this trial. The experimental design, lotting and feeding regimes were the same as described in trial I except that: (1) oxytetracycline was fed at the rate of 70 mg. per head daily; (2) corn silage was fed at the rate of 15 lb. per head daily the first 126 days of the trial.

Results and Discussion

Production and Carcass data are shown in tables 1 and 2. Combined average daily gains, carcass grades and marbling scores are given in table 3. Mean squares from the analysis of variance of the pooled data of trials I and II are included in table 4.

The average daily gains of steers and heifers fed pelleted hay-ground ear corn were 14.2% and 8.7% greater, respectively, than their controls. There was a significant interaction ($P<.01$) for weight gain of pelleted hay with ground ear corn in the heifer trial. Weight

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TABLE 1. THE EFFECT OF FEEDING PELLETED HAY, PELLETED EAR CORN AND A TRANQUILIZER TO FATTENING STEERS OCTOBER 8, 1958–MARCH 1, 1959 (144 DAYS). TRIAL I

Lot no.	1, 2	3, 4	5, 6	7, 8	1, 3, 5, 7	2, 4, 6, 8
					Hydroxyzine comparison	
Treatments	Baled hay-Ground ear corn	Pelleted hay-Ground ear corn	Baled hay-Pelleted ear corn	Pelleted hay-Pelleted ear corn	Control	Hydroxyzine
No. of steers[a]	16	15	16	16	31	32
Initial wt., lb.	860	866	862	861	866	859
Final wt., lb.	1123	1168	1141	1137	1151	1133
Av. daily gain, lb.	1.83	2.09	1.94	1.91	1.98	1.90
Change, %	....	+14.2	+6.0	+4.4	+4.2	....
Av. daily feed, lb.[b]						
Ground ear corn	15.33(13.34)	15.78(13.96)	14.64(12.94)	13.82(12.22)	14.95(13.19)	14.80(13.02)
Linseed oil meal	1.45(1.30)	1.45(1.30)	1.45(1.30)	1.44(1.29)	1.44(1.30)	1.44(1.30)
Alfalfa-brome hay	3.99(3.47)	4.00(3.61)	3.97(3.44)	3.95(3.57)	3.99(3.53)	3.97(3.51)
Corn silage	8.49(2.15)	8.53(2.16)	8.50(2.15)	8.38(2.12)	8.49(2.15)	8.46(2.14)
Change, %[c]	....	+3.8	−2.1	−5.2	....	....
Feed per cwt. gain, lb.[b]						
Ear corn	838(729)	753(667)	755(668)	723(639)	755(666)	778(684)
Linseed oil meal	79(71)	69(62)	75(67)	75(68)	73(66)	75(68)
Alfalfa brome hay	218(190)	191(173)	205(178)	207(187)	202(178)	208(185)
Corn silage	464(117)	407(103)	439(111)	438(111)	429(109)	445(113)
Change, %[c]	...	−9.2	−7.5	−9.2	...	+3.0
Carcass grade[d]	9.13	9.63	8.81	9.63	9.52	9.06
Marbling score[e]	5.56	5.88	5.44	5.87	5.85	5.53

[a] One steer removed from lot 3 because of bloat.
[b] Figures in brackets show moisture-free feed.
[c] Percent change based on moisture-free feed.
[d] Carcass grades: 8, good; 9, high good; 10, low choice.
[e] Marbling scores: 5, small; 6, modest; 7, moderate.

TABLE 2. THE EFFECT OF FEEDING PELLETED HAY, PELLETED EAR CORN AND A TRANQUILIZER TO FATTENING HEIFERS MARCH 20–SEPTEMBER 29, 1959 (191 DAYS). TRIAL II

Lot no.	15, 16	17, 18	19, 20	21, 22	15, 17, 19, 21	16, 18, 20, 22
					Hydroxyzine comparison	
Treatments	Baled hay-Ground ear corn	Pelleted hay-Ground ear corn	Baled hay-Pelleted ear corn	Pelleted hay-Pelleted ear corn	Control	Hydroxyzine
No. of heifers[a]	16	16	15	15	32	30
Initial wt., lb.	574	574	575	574	574	574
Final wt., lb.	924	955	971	922	940	945
Av. daily gain, lb.	1.83	1.99**	2.07**	1.83	1.92	1.95
Change, %	....	+8.7	+13.1	0	....	....
Av. daily feed, lb.[b]						
Ground ear corn	10.08(8.60)	10.98(9.37)	11.09(9.79)	10.43(9.21)	10.61(9.05)	10.68(9.26)
Linseed oil meal	1.48(1.31)	1.48(1.31)	1.48(1.31)	1.48(1.31)	1.48(1.31)	1.48(1.31)
Alfalfa brome hay	3.98(3.49)	3.97(3.54)	3.94(3.46)	3.95(3.51)	3.96(3.51)	3.96(3.50)
Corn silage	9.40(2.57)	9.44(2.58)	9.36(2.56)	9.40(2.57)	9.39(2.57)	9.41(2.58)
Change, %[c]	....	+5.2	+7.2	+3.9	....	....
Feed per cwt. gain, lb.[b]						
Ground ear corn	551(470)	551(470)	535(472)	569(503)	553(472)	548(476)
Linseed oil meal	81(72)	74(66)	71(63)	81(71)	77(68)	76(67)
Alfalfa-brome hay	217(191)	199(178)	190(167)	216(192)	207(183)	204(180)
Corn silage	513(141)	474(130)	451(124)	514(141)	490(134)	483(132)
Change, %[c]	...	−3.4	−5.5	+3.8	...	...
Carcass grade[d]	9.13	9.81**	8.41	9.44**	9.22	9.18
Marbling score[e]	5.81	6.50**	5.21	6.08**	5.94	5.86

[a] One heifer removed from each of lots 20 and 22 because of prolapse.
[b] Figures in brackets show moisture-free feed.
[c] Percent change based on moisture-free feed.
[d] Carcass grades: 8, good; 9, high good; 10, low choice.
[e] Marbling scores: 5, small; 6, modest; 7, moderate.
** Average daily gains interaction of pelleted hay with ground ear corn and baled hay with pelleted ear corn highly significant ($P<0.01$).
** Carcass grade differences due to baled hay vs. pelleted hay highly significanct ($P<0.01$).
** Marbling score differences due to baled hay vs. pelleted hay highly significant ($P<0.01$), and ground ear corn vs. pelleted ear corn significant ($P<0.05$).

TABLE 3. COMBINED AVERAGE DAILY GAINS, CARCASS GRADES AND MARBLING SCORES FOR TRIALS I AND II

Treatments	Baled hay-Ground ear corn	Pelleted hay-Ground ear corn	Baled hay-Pelleted ear corn	Pelleted hay-Pelleted ear corn	Hydroxyzine comparison	
					Control	Hydroxyzine
No. of cattle	32	31	31	31	63	62
Average daily gain, lb.	1.83	2.04*	2.00*	1.87	1.95	1.92
Carcass grade[a]	9.13	9.72**	8.61	9.53*	9.38	9.12
Marbling score[b]	5.69	6.19**	5.32	5.98**	5.89	5.70

[a] Carcass grades: 8, good; 9, high good; 10, low choice.
[b] Marbling scores: 5, small; 6, modest; 7, moderate.
* Average daily gain interaction of pelleted hay with ground ear corn and baled hay with pelleted ear corn significant ($P<0.05$).
** Carcass grade differences due to baled hay vs. pelleted hay highly significant ($P<0.01$) and ground ear corn vs. pelleted ear corn significant ($P<0.05$).
** Marbling score interaction due to pelleted hay with ground ear corn and baled hay with pelleted ear corn highly significant ($P<0.01$).

gains of one or two steers in each lot were subnormal. This variability resulted in non-significant differences in weight gains though the trend was similar to the treatment effect obtained in the heifer trial. The pooled data of both trials (table 3) showed a significant ($P<.05$) interaction of pelleted hay with ground ear corn as measured by average daily gains. Efficiency of feed use of the pelleted hay-ground ear corn groups was increased 9.2% and 3.4% in trials I and II, respectively, when compared to their controls. Daily feed intake was 3.8% and 5.2% greater for the pelleted hay-ground ear corn groups in trials I and II, respectively, than the controls. This greater feed intake may have been responsible for the greater weight gains of these groups. Boren *et al.*, (1959) fed weanling heifer calves sorghum silage and 5 lb. of either baled or pelleted alfalfa hay. Heifers fed pelleted hay had 20% greater average daily gain and were 16% more efficient in their feed utilization than the controls. Their results showed slightly increased feed intake due to feeding a limited amount of pelleted hay.

TABLE 4. ANALYSIS OF VARIANCE, TRIALS I AND II COMBINED

Source[a]	d.f.	Mean squares		
		Daily gains	Carcass grades	Marbling scores
Treatments (T)	3	0.3380*	7.7078**	4.5382*
BH vs. PH	1	0.0563	18.3770**	10.7532*
GEC vs. PEC	1	0.0000	3.8851	2.6738
Interaction	1	0.9574*	0.8613	0.1876
Tranquilizer (Q)	1	0.0228	2.0757	1.2207
TXQ	3	0.0020	1.4369	0.4868
Replicate (R)	1	0.0048	0.3101	1.4239
RXT[b]	7	0.1053	0.6332	0.8929
Within	109	0.0668	1.4686	0.6167
Total	124			

[a] BH, baled hay; PH, pelleted hay; GEC, ground ear corn; PEC, pelleted ear corn.
[b] Used as error term in F test.
* ($P<0.05$).
** ($P<0.01$).

Steers fed baled hay-pelleted ear corn gained 6% faster with 7.5% more efficient utilization of feed than the controls. Heifers, in a similar comparison, gained 13.1% faster with 5.5% more efficient utilization of feed. There was a significant ($P<.01$) interaction between baled hay and pelleted ear corn in the heifer trial. When the data from both trials were combined, this interaction was significant ($P<.05$). Because of the variability of weight gains the interaction between baled hay and pelleted ear corn was not significant in the steer trial. However, the data show that the trend in steer weight gains was similar to that of the heifers. Daily feed intake was variable; it decreased slightly in trial I and increased in trial II as a result of feeding pelleted ear corn in combination with baled hay.

The average daily gains of the pelleted hay-pelleted ear corn group (steers and heifers) were similar to their controls. Their daily feed intake was 5.2% less in trial I and 3.9% more in trial II than their controls. Efficiency of feed use was variable.

Webb and Cmarik (1958a) fed yearling steers a complete pelleted ration that contained 65% ground ear corn and compared it with the same ration in the meal form. Corn silage was fed also. The cattle fed pellets gained 2.75 lb. per head daily compared to 2.58 lb. for the meal-fed cattle. Daily feed intake was slightly lower for the group fed pellets, but they utilized their feed more efficiently. However, in other experiments where relatively high concentrate rations were pelleted and fed to beef cattle the results have indicated that weight gains have not been improved (Burroughs *et al.*, 1959b; Perry *et al.*, 1959; Weir *et al.*, 1959; McCroskey

et al., 1960). Efficiency of feed use has often been enhanced by feeding pelleted complete high concentrate or high roughage rations (Webb and Cmarik, 1958a; Weir *et al.*, 1959; McCroskey *et al.*, 1960).

Statistical analysis of carcass grades of heifers showed that there was a significant ($P<.01$) difference between the groups fed baled hay and those fed pelleted hay. The marbling scores of carcasses from heifers fed pelleted hay were significantly ($P<.01$) higher than from heifers fed baled hay. A similar analysis of carcass grades of steers showed no significant differences although the trend in grades of the steers was similar to that of the heifers. Analysis of the combined data (table 4) showed that the difference between baled hay and pelleted hay was significant ($P<.01$). The groups fed ground ear corn had significantly higher carcass grades ($P<.05$) than those fed pelleted ear corn. Marbling scores of the carcasses from heifers fed ground ear corn were significantly ($P<.05$) higher than those from the heifers fed pelleted ear corn. Marbling scores of the steer carcasses were not significantly different. They did, however, have the same trend in response to treatments as the heifers. When the data were combined the marbling scores of the carcasses from cattle fed pelleted hay were significantly ($P<.05$) higher than those from cattle fed baled hay.

In these trials the feeding of a limited amount of hay (4 lb. per head daily) in pellet form consistently increased carcass grade and marbling score. Likewise, carcasses from cattle fed ground ear corn graded higher than carcasses from cattle fed pelleted ear corn. The reasons for the above results are not known. Results of other investigations have shown no consistent differences in the carcass grades of cattle fed either baled or pelleted hay and/or complete meal or pelleted rations (Webb *et al.*, 1958a; Burroughs *et al.*, 1959b; McCroskey *et al.*, 1960).

In these trials, feeding 2.5 mg. hydroxyzine daily to fattening cattle had little or no effect upon feed lot performance or carcass characteristics. These results are essentially in agreement with those of Beeson *et al.* (1959), Burroughs *et al.* (1959a) and Perry *et al.* (1960), but not with those of Ralston and Dyer (1959) or Sherman *et al.* (1959).

Summary

Two experiments with 125 beef cattle were conducted to study the effects of feeding: (1) a limited amount of either baled or pelleted hay; (2) ground or pelleted ear corn; (3) a tranquilizer.

The interactions of pelleted hay with ground ear corn and baled hay with pelleted ear corn were significant ($P<.05$) with respect to weight gains. Cattle fed pelleted hay had significantly higher carcass grades ($P<.01$) and marbling scores ($P<.05$) than cattle fed baled hay. Cattle fed ground ear corn had significantly higher ($P<.05$) carcass grades than cattle fed pelleted ear corn. Feeding a tranquilizer had no effect upon feed lot performance or carcass characteristics.

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DIGESTIVE ENZYME DEVELOPMENT IN THE YOUNG PIG [1]

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THE development of early weaning as a practical aspect of pork production has opened new areas of research in the evaluation of the nutritional requirements of the young pig. These studies have been hampered by inadequate knowledge of factors which influence the nutrition of the neonatal pig (Catron *et al.*, 1957). Attempts were made, therefore, to gather certain fundamental data concerning factors which have a direct bearing on the performance of the young pig. The results of preliminary experiments on the digestive enzymes of the baby pig were reported previously (Hudman *et al.*, 1957; Lewis *et al.*, 1957). More recent findings are detailed herein, together with a discussion of these results in the light of nutritional studies which have been previously reported.

Materials and Methods

Animals. Sixteen litters of crossbred pigs were randomly alloted to two treatments. Eight litters nursed their dams throughout the experimental period; the other eight litters after weaning at 1 week of age ate self-fed dry rations.

The weaned pigs were housed in individual pens on concrete with wood shavings for bedding. They received a ration containing 40% dried skim milk (DSM) from 1 to 2 weeks of age, a 20% protein, corn-soybean oil meal (SBOM) starter diet until 5 weeks of age and an 18% protein, corn-SBOM grower diet through the remaining experimental period (table 1).

The sows with nursing litters were maintained in individual pens on concrete exterior to the building which housed the weaned pigs. Twice-a-day the sows were removed from the pens and fed the lactation ration presented in table 1.

Each pig was given a pill containing 292 mg. of reduced iron at birth and at 7 days of age. Pigs nursing sows were given an additional iron pill each week throughout the experimental period. Water was provided by automatic founts, and pens were cleaned daily. Other management practices were similar to those reported by Speer *et al.* (1954).

Sixteen pigs were sacrificed on the day of birth and an additional sixteen each week thereafter to 8 weeks of age. Each sacrifice-group comprised two littermates from each of four litters on each treatment. Thus, two randomly-selected pigs were removed from each litter every other week. Prior to sacrifice of the pigs, the sows or feeders were removed from the pigs for a 2-hour period, were replaced for 30 minutes, then were removed again for 1 hour.

Sample Collection. One hour after the last meal, the pig was electrocuted by attaching one electrode of a 110-volt line to the ear and the other electrode to the skin near the anus for 10 to 20 seconds.

The pituitary gland, adrenal gland, kidneys, spleen, liver, lungs and heart were removed, cleaned of extraneous material, and individually weighed. The stomach and intestines were isolated and the stomach ingesta and chyme from the small intestine were expressed and collected. Material to be used for enzyme determinations was immediately frozen with dry ice and stored at −15° C. until assayed.

Sample Assays. The stomach was weighed, then the entire organ, or a representative sample thereof, was homogenized for 2 minutes in a Waring semimicro or liter blendor container with 9 times the sample weight of 0.085 *N* HCl and stored in a covered flask for 16 to 20 hours at 5° C. Proteinase activities were measured by a milk clot procedure modified after Anderson *et al.* (1951).

Stomach ingesta were homogenized for 1

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TABLE 1. COMPOSITION OF DIETS

Ingredient	Age fed (wk.) 2–3	3–5	5–8	Lactation
Ground yellow corn	9.55	21.1	69.0	40.05
Ground oats	.....	20.0	.....	20.0
Sucrose	10.0	15.0	.....	.....
Dextrose	5.0	.....	.....	.....
Solvent soybean oil meal (50%)	17.5	15.0	20.0	10.0
Meat and bone scraps	.....	.....	.....	5.0
Dried skimmilk	40.0	10.0	.....	.....
Condensed fish solubles	2.5	2.5	2.5	2.5
Dried whey (70% lactose)	2.5	10.0	2.5	5.0
Dried distiller's solubles	.....	.....	2.5	.....
Corn steep water	1.0	.....	.....	.....
Dehydrated alfalfa meal	.....	.....	.....	10.0
Dried brewer's yeast	1.0	.....	.....	.....
Dried beet pulp	2.0	.....	.....	5.0
Stabilized lard	5.0	2.0	.....	.....
Vitamin-antibiotic premix	2.5[a]	2.0[b]	1.0[c]	1.25[d]
Calcium carbonate	0.15	0.45	0.80	.....
Dicalcium phosphate	0.60	1.30	1.0	0.50
Iodized salt	0.50	0.50	0.50	0.50
Trace mineral mix[e]	0.20	0.15	0.20	0.20

[a] Contributed per pound of diet: vitamin A, 1000 I.U.; vitamin D_3, 1000 I.U.; riboflavin, 5 mg.; Ca pantothenate, 10 mg.; niacin, 30 mg.; choline chloride, 500 mg.; vitamin B_{12}, 20 mcg.; d-alpha tocopherol acetate, 10 mg.; ascorbic acid, 300 mg.; menadione, 1.0 mg.; thiamine HCl, 2.0 mg.; pyridoxine, 2.0 mg.; para amino benzoic acid, 8.0 mg.; folic acid, 9 mg.; chlortetracycline, 25 mg.; penicillin, 12.5 mg. and streptomycin, 12.5 mg.

[b] Contributed per pound of diet: vitamin A, 2800 I.U.; vitamin D_3, 600 I.U.; riboflavin, 1.4 mg.; Ca pantothenate, 2 mg.; niacin, 18 mg.; vitamin B_{12}, 20 mcg.; chlortetracycline, 50 mg.

[c] Contributed per pound of diet: vitamin A, 1800 I.U.; vitamin D_3, 300 I.U.; Ca pantothenate, 2 mg.; niacin, 8 mg.; vitamin B_{12}, 10 mcg.; chlortetracycline, 25 mg.

[d] Contributed per pound of diet: vitamin D_3, 500 I.U.; riboflavin, 1 mg.; Ca pantothenate, 5 mg.; niacin, 13 mg.; choline chloride, 30 mg.; vitamin B_{12}, 10 mcg.; penicillin, 10 mg.

[e] 0.2% contributes per pound of diet: Fe, 64 mg.; Cu, 4.4 mg.; Co, 1.42 mg.; Zn, 74.2 mg.; Mn, 51.6 mg.

minute with acid water (1 part ingesta with 3 parts of acid water by weight) and were stored in stoppered flasks overnight (16 to 20 hours) at 5° C. The homogenate, or a dilution of the homogenate in acid water, was used for the assay of stomach proteinase, as described above.

The pancreas was homogenized for 2 minutes with 4 times its weight of 0.2% NaCl solution. Two ml. of the homogenate were mixed with 22.0 ml. of distilled water and 1.0 ml. of enterokinase (Kunitz, 1938) solution (1.0 mg. of enterokinase per ml.), then the mixture was adjusted to pH 5.6 ± 0.1 with 0.1 *N* HCl. The original and activated homogenates were held at 5° C. Tributyrinase and amylase determinations were made directly on dilutions of the original homogenate after storage for 1 and 3 days, respectively. Dilutions for amylase assays were made in 0.2% NaCl solution. Proteinase determinations were made after holding the enterokinase-tissue mixture for 2 days. Tributyrinase activities were determined by a method modified after Goldstein *et al.* (1948). Pancreatic amylase activities were estimated by measuring the increase in reducing substances, calculated as mg. of maltose, with the Somogyi (1952)—Nelson (1944) reagents and a substrate of soluble starch. Pancreatic proteinase activities were determined by a disc-plate method similar to that described by Lewis *et al.* (1957), except that four filter paper discs were used for each of three dilutions of each sample.

Intestinal contents were weighed, then an aliquot was homogenized for 1 minute with 3 times its weight of distilled water and stored in a stoppered flask at 5° C. for 5 to 7 hours before use. No activation, other than that which took place prior to and during the assays, was deemed necessary, although the effect of enterokinase on proteinase level of the intestinal ingesta was not investigated. Determinations were similar to procedures described above for the estimation of pancreatic proteinase activities.

Intestinal tissue was prepared for assay by chopping each block of frozen tissue into small pieces, then homogenizing a representative sample for 1 minute with 4 times its weight of distilled water. The homogenate was held in a stoppered flask at room temperature for 3 to 6 hours, with frequent agitation. Lactase, maltase and sucrase determinations were made by a reductimetric method similar to that used for amylase assays utilizing the appropriate substrates.

Results

Growth curves of the early weaned and nursing pigs are shown in figure 1. In comparison to the nursing pigs, the early weaned pigs grew more slowly from 1 to 4 weeks of age, at an equal rate between 4 and 5 weeks of age, and more rapidly for the remainder of the experimental period. At 7 weeks of age the body weight of the weaned pigs exceeded that of the nursing pigs.

Stomach Tissue. Proteinase activities per unit of stomach tissue (figure 2) followed trends similar to those reported in pigs (Lewis *et al.*, 1957; Braude *et al.*, 1958), rats Langendorff, 1879), cats (Sewall, 1878; Lim, 1922; Werner, 1948) and rabbits (Werner,

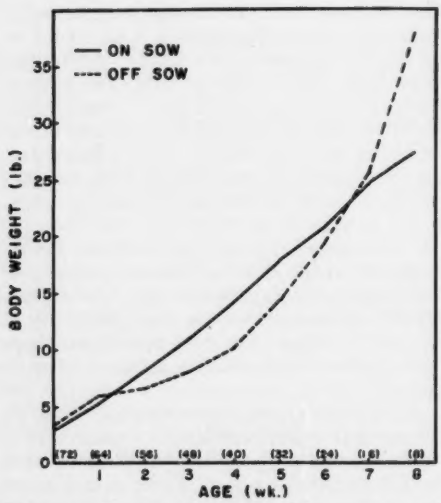

Figure 1. Growth curves of early weaned and nursing pigs.

1948). Gastric proteinase was recovered from tissues of the newborn pig, although Langendorff (1879) did not observe such an occurrence. There were little or no increases in activity during the first 2 weeks after birth. A rapid increase in activity started at 3 weeks of age and continued throughout the experimental period. No statistically significant differences in the levels of enzyme activities per gram of tissue were observed between the two diet groups. The weaned pigs, however, possessed more stomach tissue (table 2), therefore, more potential secretory capacity, which was reflected in higher levels of enzyme per unit pig weight during the 3- to 7-week age period.

A comparison of figures 2 and 3 shows that, at the time of sacrifice, the proteinase level of the stomach tissue was not directly related to the quantities of this enzyme in the stomach ingesta, especially for the weaned pigs. As would be expected, the proteinase activity per unit wet weight of ingesta was only a fraction of the activity found in the tissues.

Stomach Ingesta. Of much interest was the influence of a dry diet (table 1) on the quantities of proteinase found in the stomach ingesta of the young pig. While the proteinase activity of the stomach tissue of both nursing and weaned animals followed a similar pattern of considerable increase with age (figure 2), there was a noticeable decrease, even at 2 weeks of age, in the quantity of enzyme per unit wet weight of ingesta of the weaned animals (figure 3). When calculated on a body weight basis, the levels of stomach (ingesta) proteinases were similar in the two groups of animals at birth, and at 1 and 2 weeks of age. It would appear, therefore, that the quantity of proteinase recovered from the ingesta was not influenced by the DSM diet, but was decreased when the SBOM was fed. The above results confirm the work of Braude *et al.* (1958), except for their observation that the proteolytic activity of the stomach contents of pigs on a milk diet remained relatively constant from 4 to 36 days of age.

Several workers (Klumpp and Neale, 1930; Kvasnitskii and Bakeeva, 1940; Cunningham and Brisson, 1957) have suggested that the pH of the stomach ingesta may not be suitable

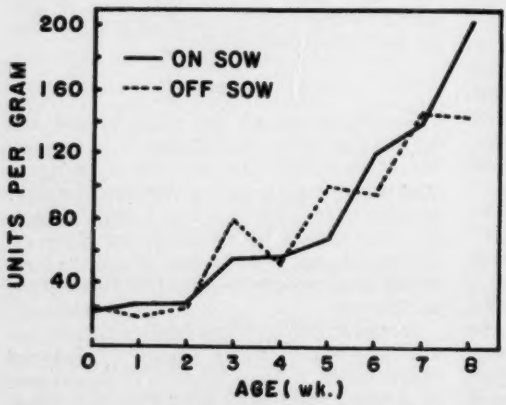

Figure 2. Proteinase activity of stomach tissue.

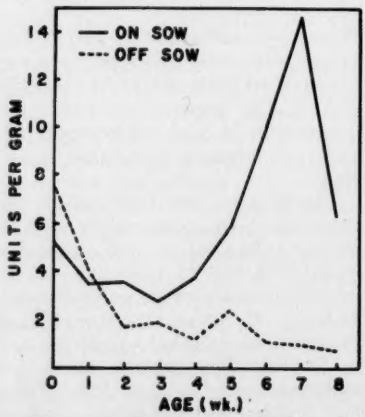

Figure 3. Proteinase activity of stomach ingesta.

TABLE 2. SUMMARY OF PERFORMANCE, ORGAN WEIGHTS AND WEIGHTS OF CONTENTS OF DIGESTIVE TRACT

Item	Age (weeks)								
	0	1	2	3	4	5	6	7	8
Body weight of pigs sacrificed, lb.									
On sow	2.8	5.9	8.4	11.9	12.6	21.2	19.0	24.0	27.3
Off sow	2.8	6.1	7.8	9.9	11.7	17.9	19.5	22.8	32.6
Feed/gain									
Off sow	...	...	1.40	3.02	2.45	2.07	1.98	2.31	2.02
Thyroid weight, mg.									
On sow	182	278	362	550	597	927	837	1009	1197
Off sow	198	332	420	424	528	850	885	1190	2285
Pituitary weight, mg.									
On sow	16	27	34	40	51	62	57	56	69
Off sow	15	22	36	27	54	49	61	56	78
Pancreas weight, gm.									
On sow	2.0	4.3	5.9	9.8	10.8	18.9	18.6	25.5	25.4
Off sow	1.8	4.4	7.8	8.3	13.0	19.7	28.4	30.7	47.9
Stomach tissue weight, gm.									
On sow	6.0	16.8	24.9	40.4	43.3	57.9	62.0	79.4	81.7
Off sow	5.5	16.5	31.0	36.8	53.6	66.3	108.0	103.4	153.2
Intestinal tissue weight, gm. Anterior third of small intestine									
On sow	13.1	29.2	31.4	63.2	68.3	106.9	102.4	129.2	138.0
Off sow	12.3	29.7	40.7	58.7	83.4	100.9	154.2	157.9	203.2
Intestinal tissue weight, gm. Middle third of small intestine									
On sow	11.2	27.1	25.8	55.7	65.6	101.1	92.0	107.4	114.4
Off sow	13.9	28.6	44.1	59.1	91.8	90.0	140.8	147.1	211.2
Intestinal tissue weight, gm. Posterior third of small intestine									
On sow	13.3	29.3	43.7	71.4	80.1	100.1	125.7	121.2	131.6
Off sow	13.3	36.2	41.6	60.7	79.5	92.1	157.9	167.8	232.6
Stomach ingesta, gm.									
On sow	6.4	18.2	13.0	23.6	25.1	31.9	70.8	83.9	147.9
Off sow	9.7	14.1	21.1	42.0	80.5	69.6	177.6	155.4	189.3
Intestinal ingesta, gm. Anterior third of small intestine									
On sow	2.1	8.7	11.6	19.1	14.0	23.1	36.4	38.8	40.0
Off sow	1.3	9.4	12.2	24.0	29.5	51.5	50.6	51.7	68.4
Intestinal ingesta, gm. Middle third of small intestine									
On sow	1.5	10.3	9.0	24.2	13.9	30.8	26.1	39.5	45.2
Off sow	1.2	8.0	28.8	29.4	50.6	42.5	54.4	49.2	71.3
Intestinal ingesta, gm. Posterior third of small intestine									
On sow	1.4	5.6	13.5	20.9	23.6	25.9	48.5	49.7	51.0
Off sow	2.1	6.7	28.7	45.6	48.7	70.1	64.6	55.3	78.5

for gastric proteinase activity in the young animal; but, examination of the ingesta of pigs used in these (table 3) and previous (unpublished) studies has not implicated a change in acid secretion associated with improved utilization of plant protein as the pig increases in age.

Pancreas Tissue. Tissue tributyrinase activities found in the pancreases of unweaned pigs (figure 4) were comparatively high at birth and displayed a gradual increase with age. These data support the studies of others with pigs (Kitts *et al.*, 1956), the human (Klumpp and Neale, 1930; Andersen and Dueholm, 1949; Berkman, 1951; Carnevale *et al.*, 1954) and the rat (Tuba and Hoare, 1951), but do not agree with the results of Kryuchkova (1939) on dogs. In addition, a distinct litter effect was noted on lipase level resulting in the bi-weekly ascent of the curve (figure 4). The litters which contained higher levels of pancreatic tributyrinase were also faster gaining litters (figures 1 and 4). Kitts *et al.* (1956) concluded from their studies that

TABLE 3. HYDROGEN ION CONCENTRATION (pH) OF VARIOUS SECTIONS OF THE DIGESTIVE TRACT

Item	Age (weeks)								
	0	1	2	3	4	5	6	7	8
Stomach contents									
On sow	4.0	3.9	4.3	4.4	4.6	5.2	4.5	4.2	4.8
Off sow	4.3	4.1	4.7	4.6	4.4	3.9	3.9	3.3	4.0
Anterior third of small intestine									
On sow	6.5	6.4	6.5	6.4	6.5	6.4	6.2	6.2	6.2
Off sow	6.3	6.4	6.0	5.8	5.9	5.8	5.9	5.8	5.9
Middle third of small intestine									
On sow	6.8	6.7	6.6	6.5	6.8	6.6	6.5	6.4	6.8
Off sow	6.6	6.6	6.1	5.7	6.2	6.2	6.1	6.0	6.1
Posterior third of small intestine									
On sow	6.8	6.8	6.7	6.8	6.8	7.0	6.8	6.6	6.9
Off sow	6.7	6.8	6.4	5.9	6.4	6.3	5.9	6.1	6.2
Large intestine									
On sow	6.7	6.8	6.7	6.6	6.9	7.0	7.1	6.9	7.0
Off sow	7.0	6.6	6.8	6.6	6.3	6.2	5.9	5.7	5.9

lipid hydrolyzing enzymes were relatively abundant in the baby pig and were, therefore, of no special significance in the development of the young animal. The data in figure 4 would indicate that the significance of lipolytic enzymes in the nutrition of the baby pig is worthy of further investigation, especially if the influence of weaning on the levels of this particular enzyme complex is considered. Weaning caused a marked diminution of tributyrinase activities. Recovery appeared to begin at 4 weeks of age, and by 7 weeks of age tributyrinase levels of the weaned pigs approached those found in the unweaned animals. In fact, by 6 weeks of age, on a body weight basis (figure 4, table 2), the lipolytic activity of the pancreatic tissue of the weaned pigs exceeded that of the groups which received sows milk. The above findings may aid in the explanation of the results of some workers (cf. Lloyd and Crampton, 1957; Lloyd *et al.*, 1957), who have found that fat digestion by the weaned animal increases with age. A possible explanation might be that change of diet (weaning) caused a depression in enzyme secretion which was subsequently regained.

Tissue Amylase. Estimates of the levels of amylase per unit wet weight of pancreatic tissues in unweaned pigs (figure 5) yielded results similar to those obtained by various workers using young pigs (Kitts *et al.*, 1956; Hudman *et al.*, 1957) or human infants (Klumpp and Neale, 1930; Andersen and Dueholm, 1949; Berkman, 1951; Vazquez, 1951; Carnevale *et al.*, 1954). A significant linear regression of amylase level on age and no significant difference between on and off sows suggests that the two points at 5 and 6 weeks of age for the nursing group are grossly out of line. The animals, at 6 weeks of age, contained relatively large quantities of stomach and intestinal ingesta (table 2). Vonk *et al.* (1957) indicated that such discrepancies were to be expected unless an extended period of starvation was used and the size of the test meal was closely regulated.

A large increase in amylase activity was as-

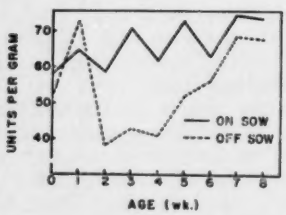

Figure 4. Tributyrinase activity of pancreatic tissue.

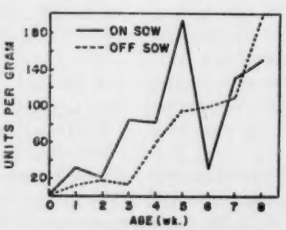

Figure 5. Amylase activity of pancreatic tissue.

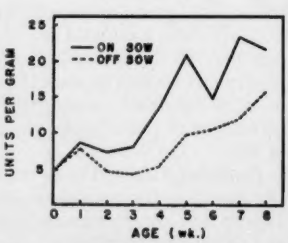

Figure 6. Proteinase activity of pancreatic tissue.

sociated with increasing age in the unweaned group, up to 5 weeks of age, after which the level was erratic (see also Hudman *et al.*, 1957). Amylolytic activity followed a pattern of linear increase with age in the weaned pigs. The average linear regression of the two curves was highly significant, but the age × treatment interaction was not significant. Kryuchkova (1939) had previously found marked changes in enzyme levels in the dog at 2 weeks of age (when control of the pancreas was supposedly taken over by the vagus nerve) and at 6 weeks of age (when the dog was weaned). No such marked changes in enzyme production were noted in these and in previous studies on the pig.

The data (units/gm. wet weight of tissue) indicate that the point of maximum amylase production had not been reached on either diet. On a pig weight basis, however, there were indications that further increases in amylase activity per unit of pancreatic tissue were being balanced, after 5 weeks of age, with comparable increases in pig weight (figure 5, table 2). It is of interest that Cunningham (1959) found no significant difference in the digestibility of raw or soluble corn starch by 3- to 10-day-old pigs *vs.* 10- to 23-day-old pigs in digestion trials. Carbohydrate tolerance curves, however, showed that ability to digest soluble corn starch increased with age, as would be expected from examination of the data shown in figure 5. Raw starch was utilized to a lesser extent than soluble starch (Cunningham, 1959).

In previous studies (Lewis *et al.*, 1957), no definite increase in tryptic activity was observed with increasing age of the baby pig. It was thought that, at birth, the pig was capable of producing relatively large quantities of trypsin. Rather high levels of tryptic activity have been noted at birth in the human (Berkman, 1951; Carnevale *et al.*, 1954). In the present studies (figure 6), a 4-fold increase in the enzyme activity per unit of wet tissue was noted during the first 8 weeks of age of the unweaned pigs. Similar results have been reported by Braude *et al.* (1958). There was a depression of pancreatic proteinase activity upon weaning of the pigs to a dry diet (figure 6), closely resembling the pattern for pancreatic lipase (figure 4), but dissimilar to the amylase pattern (figure 5). Initial recovery (increase in proteinase level) could be observed at 4 weeks of age. Even at 8 weeks of age the level of tryptic activity apparently had not reached a maximum in the weaned pigs, although again the enzyme units per kg. body weight (figure 6, table 2) suggest that the curve might taper off at a maximum level similar to that reached by the unweaned pigs.

Intestinal Ingesta. Levels of proteinase per gm. wet weight of baby pig intestinal contents increased with increasing age from birth through 7 weeks of age (figure 7). Proteinase levels were generally lower in the first third of the small intestine than in the two distal sections. The quantities of proteinase present were essentially the same in the latter two intestinal segments of the weaned pigs. The level of proteinase in the pancreatic tissues of unweaned pigs was higher than that of weaned pigs (figure 6); however, the enzyme activity recovered from the intestinal contents (figure 7) was higher in the weaned pigs, except for the third intestinal segment. Presumably, the proteinase activity should be greatest in the proximal third of the small intestine; however, the samples were activated in the absence of enterokinase other than that normally present. Vonk *et al.* (1957) and Braude *et al.* (1958) observed similar levels of proteinase activities upon examination of the intestinal and cecal chymes of the young pig, but it appeared that the above authors also neglected to examine the effect of additional enterokinase on proteinase yields.

The presence of considerable proteinase activity in the distal section of the small intestine of the baby pig, especially when a SBOM diet is fed (figure 7) indicates that the reabsorption of endogenous protein (at least of trypsin) was proportionally quite small when compared to the percentage of reabsorption (loss of recoverable activity) in the mature (250 lb.) animal. Proteinase activities of the market weight hog were diminished considerably in the latter segments of the intestinal tract (unpublished data). Levels of other digestive enzymes in the intestinal contents of baby pigs were not examined, but Vonk *et al.* (1957), using older pigs than the ones used here, found that cecal amylase levels were about as high as amylase levels in the intestinal contents.

Adaptation to any particular diet might be caused both by the quantity of a dietary constituent (Gupta *et al.*, 1958), as well as the type of protein or carbohydrate source (Magee and Anderson, 1955). For the pigs receiving milk the proteinase activity of the stomach tissue and of the intestinal tissue followed similar patterns, when examined on

a body weight basis (figures 3 and 7, table 2). For the pigs on the dry diet a considerable increase in intestinal proteinase per kg. of body weight was observed, however, little change in stomach proteinase was noted.

Intestinal Tissue. At birth, lactase was the predominant intestinal carbohydrase of the three enzymes examined (Heilskov, 1951; Bailey *et al.*, 1956; DeGroot and Hoogendoorn, 1957) (figure 8). A rapid decrease in enzyme activity occurred at 1 to 3 weeks of age and was followed by the presence of a fairly constant quantity for the remainder of the experimental period (quadratic regression significant, P=0.01). Similar to the findings of other workers (Mendel, 1906; Plimmer, 1906/1907), quantities of enzyme observed per unit wet weight of tissue were not related to diet. The enzyme level in each section was higher (P=0.05) than in the section immediately posterior, but, contrary to the findings of DeGroot and Hoogendoorn (1957), the differences were small.

Fischer and Sutton (1949) reviewed studies on adaptation to high lactose levels in the adult rat diet and postulated that lactase production was increased. Later experiments (Fischer, 1957a, b) demonstrated that enzyme activity per unit of tissue nitrogen was similar in adapted and nonadapted animals, but that lactose stimulated growth of the mucous epithelium which was reflected in increased intestinal dry weight of the lactose-fed rats. These results were neither confirmed in the present experiments on baby pigs or by those of DeGroot and Hoogendoorn (1957) on adult rats and guinea pigs.

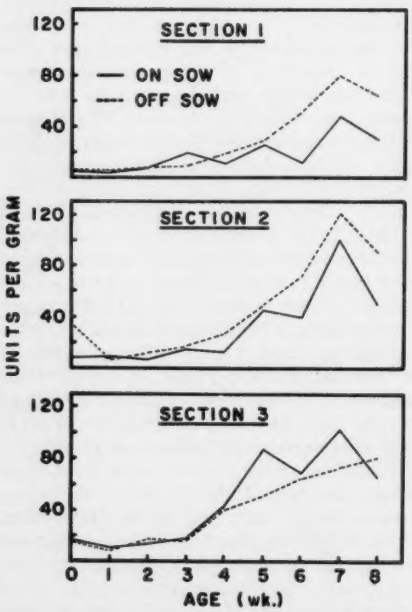

Figure 7. Proteinase activity of intestinal ingesta.

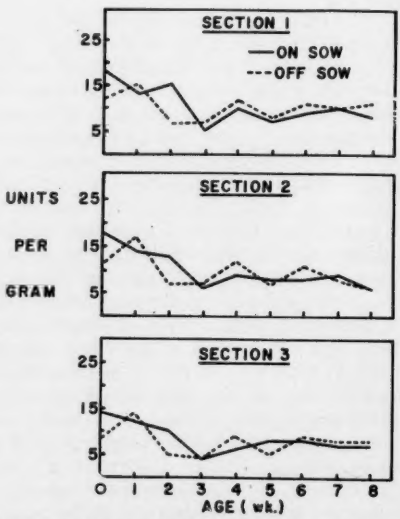

Figure 8. Lactase activity of intestinal tissue.

Quantities of maltase found per unit wet weight of tissue (figure 9) were low at birth, dropped slightly at 1 week of age, then increased gradually to 7 weeks of age. Similar trends were noted by Bailey *et al.* (1956). Activities in the first and (with 1 exception) in the second intestinal segments of the weaned pigs exceeded those of the unweaned pigs throughout the experimental period. Any induction of enzyme formation which might have accrued upon exposure of the pigs to a starchy diet took place primarily in the foremost regions of the small intestine; however, no appreciable difference in enzyme level between sections was detected.

Low levels of sucrase were present in the intestinal tissues at birth (figure 10). High

levels of the enzyme were reached at 6 or 7 weeks of age. In general, slightly higher levels of sucrase were observed in the second and third sections than in the first intestinal segment. If any adaptation occurred upon feeding of the dry diet, it was only slight.

On a body weight basis, maximum sucrase activities were about 3 and 5 times higher, respectively, than maximum maltase and lactase activities. These findings do not agree with those of Bailey *et al.* (1956), who reported rather low sucrase activities of pig

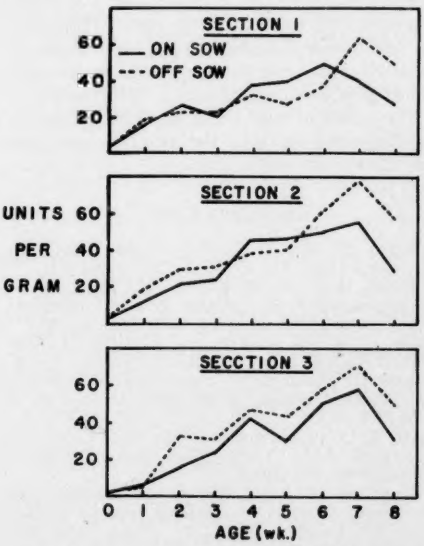

Figure 9. Maltase activity of intestinal tissue.

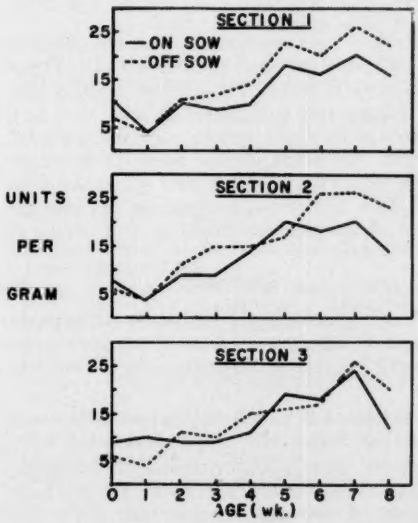

Figure 10. Sucrase activity of intestinal tissue.

intestinal mucosa relative to the quantities of maltase and lactase present. In the present studies, the sucrase activity exceeded the level of other carbohydrases examined in homogenized whole tissue by 4 weeks of age.

Discussion

Levels of most of the enzymes examined had reached maxima by the time the pigs were 7 weeks of age. Decreased enzyme levels were noted in some instances in the 8-week-old animals. These, and other data, indicate that the mature pig does not have higher levels of certain digestive enzymes in the gastrointestinal chymes than does the one- to two-month old pig. Enzymatic aspects of digestion are correlated, at least in part, with poorer utilization of plant proteins than milk proteins by the neonatal animal, but do not serve to completely elucidate the reasons for better utilization of certain proteins by the older animal than by the one-month-old animal. Enzymatic predigestion of proteins does not appear to affect the nutritive value of some proteins for the adult (Chow, 1948; Longenecker and Hause, 1958). Feed hydrolyzates have given favorable results in baby pig trials, yet, positive results were not obtained in every experiment (Catron *et al.*, 1957). Hydrolysis of proteins or carbohydrates may not be the only rate-limiting factor in the utilization of complex carbohydrates and plant proteins for the baby pig. Certainly, a lower rate of soya protein utilization compared with milk protein cannot be offset by increasing the protein level (substrate concentration) fed the baby pig (Hays, 1957; Hays *et al.*, 1959). Possibly the older pig possesses a more complete enzyme system including enzymes not differentiated by the methods so far employed (Knox *et al.*, 1956). The prevalence of other hydrolytic enzymes, (such as the nucleases), and permeases (Cohen and Monod, 1957; Cohn, 1957), should be examined in the neonatal animal. The effects of various diets on the intestinal flora (Wilbur, 1959),

excretory patterns of the animal (Widdowson *et al.*, 1958) and tissue supplies of various dietary constituents (Longenecker and Hause, 1958; Frame, 1958) should not be overlooked.

The rate of food passage also cannot be excluded as a factor affecting the nutritive value of feeds for the neonatal animal (Baker, 1959). Feeds apparently have a slower rate of passage through the digestive tract of the older pig (Castle and Castle, 1956, 1957). In addition, the nature of the diet appears to influence the rate of food passage as well as intestinal absorption (Rosenthal and Nasset, 1958).

Table 2 contains data on the wet weights of the pituitary and thyroid glands. These data were collected for another purpose, yet the information is of potential value to others interested in the physiology of the neonatal animal. No attempt was made to correlate these data with the other data presented here, and such a discussion would be beyond the scope of this paper.

Summary

A comparative study was made of the levels of certain digestive enzymes in weaned *vs.* unweaned pigs, from birth through 8 weeks of age.

Proteinase activities of the stomach tissue were low during the first 2 weeks, but later increased rapidly with age in both treatment groups. Proteinase activities of the stomach ingesta of the pigs nursing their dams decreased to 3 weeks of age, and then increased to 7 weeks of age. On the other hand, the proteinase activities of the stomach ingesta decreased throughout the experimental period in the early weaned pigs.

Pancreatic tributyrinase activities were relatively high in the unweaned animals at birth and gradually increased with age. Early weaning resulted in an initial decrease in tributyrinase levels which were not offset by subsequent increases in enzyme production until the pigs had reached 6 weeks of age. Pancreatic amylase activities increased similarly with age in both diet groups. Pancreatic proteinase activities also increased with age in both diet groups; however, enzyme concentrations in the tissues of the weaned animals were lower than levels found in unweaned animals.

Changes in the proteinase activities of the ingesta of the small intestine were similar in both treatment groups. In general, there was an increase in proteinase activity up to 7 weeks of age, with greater activity in the distal two-thirds of the intestine.

Both groups of animals exhibited similar patterns of development in levels of carbohydrases of the small intestine. There were rapid decreases in lactase levels up to 3 weeks of age, after which rather constant levels were found during the remainder of the experimental period. Following the first week of age, maltase activities increased gradually up to 7 weeks of age. Sucrase activities increased from birth through 7 weeks of age.

The results of the enzyme assays and pH measurements made on contents of the digestive tract were discussed in the light of previous studies made on young monogastic animals and present knowledge relative to the nutrition of the neonatal pig. Data on feed utilization and the wet weights of the pituitary and thyroid glands are included.

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THE METABOLISM OF ESTROGENS IN THE EWE [1]

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IN an earlier paper (Wagner *et al.*, 1960) we reported on the use of estrogens in a hormone sequence intended to bring about estrus in the anestrous ewe. It soon became evident that more information is needed about the transport of estrogens in the blood together with their metabolism and excretion before practical treatment could be made fully effective. Observations made in this study also emphasize the need for information on the influence of the physical state of administered estrogen, whether in crystalline or soluble form, on its metabolism and excretion.

Materials and Methods

The experimental animals used in this study included cycling western ewes, anestrous western ewes, Hampshire rams, and pregnant Hampshire ewes. The cycling ewes were bled during the first 12 hours of estrus and the pregnant ewes during the fourth month of gestation. One-hundred-ml. samples were taken in all cases. Systemic blood was obtained from the jugular vein. A method was also devised for collecting blood continuously from an ovarian vein. In preparation for the surgical procedure, promazine hydrochloride (Sparine, Wyeth Laboratories) was given intravenously and the operative site was infiltrated with approximately 60 ml. of 2% procaine. The ovary was exposed through an incision in the paralumbar fossa. Two of the three ovarian veins were ligated and a 2-foot length of PE 60 polyethylene tubing was inserted in the third vein. This tubing was rinsed with heparin solution, and just prior to its insertion the ewe was given 0.5 gm. heparin intravenously. This dosage was repeated one hour later.

Estradiol (0.05 to 0.5 mg.) was given intravenously to the anestrous ewes and 100 ml. blood samples taken from 9 minutes to 24 hours after injection. One mg. of estradiol was given intravenously to each of two ewes and all the urine collected by means of an anchored catheter over a period of 8 hours. The bladder was rinsed at various intervals and the rinse added to the urine sample for that time period.

The extraction procedure used was designed to remove and activate all of the estrogenic substances from whole blood. No attempt was made to separate the various metabolites of estradiol or to determine the fraction of total estrogens present in the free, conjugated, or protein-bound form. The experimental results are divided into two groups because the initial extraction of estrogens from the blood (Step A, fig. 1) was changed midway through the study due to the inadequacy of the initial extraction procedure when applied to blood containing estrogen that had been added *in vivo*.

It has been reported (Smith and Smith, 1934; Bischoff *et al.*, 1951; Diczfalusy, 1953; Diczfalusy and Lindkvist, 1956) that repeated extraction of whole blood and placental tissue with 95% ethyl alcohol will quantitatively remove the estrogens, including their conjugates. When this method was employed for the extraction of estradiol added to rams' blood *in vitro* it was very satisfactory. However, estrogen added to the blood *in vivo*, was not recoverable by this procedure, indicating that a large percentage was either bound to the protein or removed from the blood. Therefore, a mild alkaline hydrolysis was employed. An equal volume of 2 N sodium hydroxide solution was added to whole blood and the mixture was allowed to stand at room temperature for a period of 12 hours. This initial step coupled with the standard procedure employed for the remainder of the extraction gave adequate recoveries of estradiol added to rams' blood *in vitro* and was comparable to the initial extraction procedure using 95% ethyl alcohol. However, the alkaline hydrolysis also enabled the extraction of estradiol when added in the water-soluble form to rams' blood *in vivo*. Diczfalusy (1953) discussed the rationale for much of the extraction procedure described in figure 1.

The procedure used for urine was the same

[1] Contribution from the department of Physiology and Pharmacology. The data herein are taken from a thesis presented by the senior author to the School of Advanced Graduate Studies, Michigan State University, in partial fulfillment of the requirements for the Ph.D. degree in Physiology.
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A. 100 ml. of whole ewes' blood
plus
100 ml. of 2N NaOH
overnight at room temperature

B. Extract 4 times with 50:50 alcohol-ether mixture

C. Evaporate the alcohol-ether in a closed condenser system under a vacuum and under N_2

Protein residue, discard.

Discard alcohol-ether

D. Dilute water residue to 200 ml. with dist. water and add 30 ml. of con. HCl. Reflux for 1 hr. under N_2

E. Extract 1 time with 150 ml. toluene
Extract 3 times with 100 ml. ethyl ether

F. Evaporate the ether as in step C.

Discard ether

G. Extract the toluene 3 times with 60 ml. of 1.25 N NaOH

H. Adjust pH of NaOH to 5 ± 0.2 with con. HCl and extract 4 times with 100 ml. ethyl ether

Discard toluene

Discard NaOH residue

I. Wash ether 4 times with 40 ml. of dist. water.

J. Evaporate ether to dryness on slide warmer under hood at 37° C.

Discard water wash

K. Take up residue in 20 ml ether add cottonseed oil and evaporate ether as in J.

Figure 1. Flow sheet depicting the extraction of the total estrogenic activity from whole ewes' blood.

as that used for blood except that steps A through C of the extraction were omitted. In most cases this procedure was adequate, giving an extract acceptable to the assay animal. However, the extract from a few samples was very toxic. Brown (1955) reported a procedure in which toxicity could be removed by a steam distillation technique. This was not done with these samples, however, because enough of them were free of toxicity to give an adequate picture of the excretion of estrogen.

The bioassay of estrogenic activity proposed by Rubin *et al.* (1951) was used for all blood and urine extracts. Our untreated control mice did not show significant correlation between uterine weight and body weight. Therefore, the data were expressed as uterine weight without any corrections for body weight. The experimental design included one dosage level of each unknown compared against a 2-point standard curve, with five observations at each dosage level of the unknown and seven to ten at each level of the standard, depending upon the number of unknowns being assayed. This experimental design was described by Bliss (1952).

Results and Discussion

The Extraction of Estrogens from Ewes' Blood. The first part of the study was designed to determine whether assayable quantities of estrogenic substances could be extracted from 100-ml. samples of blood taken from ewes during estrus or pregnancy. The standard estrogen solutions for the assay curve were made by adding various amounts of estradiol to 100 ml. of rams' blood, extracting, and dissolving in cotton seed oil. The unknowns were extracted by the same method, and their estrogenic potency was calculated directly from the standard curve. The number of mice used for each unknown and the sensitivity of the assay was such that 0.10 to 0.20 μg. of estradiol or estradiol equivalent per 100 ml. of blood was necessary to show concentrations significantly different from zero. Therefore, 0.015 to 0.03 μg. per assay animal was

needed to give a significant uterine response. The extraction procedure was the same as outlined in the methods section except that the preliminary alkaline hydrolysis was employed for only the ovarian vein blood.

Except for one ewe, bled during the first 12 hours of natural estrus, no estrogen activity was found in the blood from an endogenous source. Blood samples from 13 cycling ewes and 13 ewes during induced estrus contained no measurable quantities of estrogen. No estrogenic activity was found in the blood collected from three ewes during their fourth month of pregnancy. Samples of ovarian vein blood were collected continuously over a 2-hour period at the rate of 20 drops a minute from PMS-stimulated ovaries of two ewes which contained at least three large follicles. Even though blood flow from the ovary was reduced and only one of the ovaries was being bled, it still could be expected that blood from this source would contain a higher concentration of estrogens than any other blood source in the body. However, none was found. Edgar (1958) using relatively small samples of blood, was able to find progesterone in ovarian vein blood of the ewe and none in peripheral blood regardless of the stage of the estrous cycle. It can only be concluded from the present results that the estrogen content of the ovarian blood of the ewe is below the limits of sensitivity of the assay method employed.

Since endogenous estrogens were not detected in the blood of the ewe, the next study was designed to determine the biological half life in the blood of an intravenously injected dose (0.2 to 0.5 mg.) of a crystalline suspension of estradiol. However, significant amounts of estrogen were not found in the blood even for samples collected as early as 5 minutes after injection.

It seemed probable that the microcrystalline suspension of estradiol might be handled differently by the ewe than a water soluble form of the hormone. Estradiol was rendered water soluble, therefore, by forming its sodium salt. A study was designed to determine the ability of various procedures to extract estrogenic substances from blood when estradiol was added *in vivo* in its two physical forms.

Three variations of the initial extraction procedure were used to determine the effect of protein hydrolysis and various solvents on the extraction of estrogens from whole blood (table 1). The data in table 1 indicate that the microcrystalline estradiol was removed from the blood stream *in vivo* to the extent that none could be detected by the methods employed. In view of the rapid disappearance of the comparatively large quantities of microcrystalline estradiol given, it is suggested that phagocytosis of these crystals might have occurred. Thus, the whole reticulo-endothelial system would serve as a depot from which the estradiol could be drawn at a rate dependent upon crystal size and solubility. This would account for its greater effectiveness over a longer period of time in producing estrous behavior as compared to the sodium salt of estradiol (Wagner *et al.*, 1960.)

When 200 to 500 μg. of the sodium salt was injected intravenously, significant quantities were found in 100 ml. samples of blood taken 5 to 9 minutes after injection, provided that the sample was subjected initially to a mild alkaline hydrolysis. The indications are that most of the estrogen was bound in some manner in a very short time after injection. The sodium salt of estradiol remained in the blood in assayable quantities longer than the crystalline estradiol. This allowed for blood samples to be taken at reasonable periods

TABLE 1. INFLUENCE OF EXTRACTION METHOD AND PHYSICO-CHEMICAL STATE ON RECOVERY OF INTRAVENOUSLY INJECTED ESTRADIOL FROM BLOOD [a]

	Crystalline suspension			Sodium salt		
Initial extraction	Amt. inj. (μg)	Conc. in blood (μg%)	No. obs.	Amt. inj. (μg)	Conc. in blood (μg%)	No. obs.
A. 4 x with ethyl alcohol, 95%	300	0	20	300	0.17*	1
B. 4 x with 1:1 ethyl alcohol-ether mixture	300	0	5	300	0	1
C. Hydrolyzed overnight with 2N NaOH	300	0	8	100	0	2
and extracted as in B	...	..	..	200	0.08*	1
	...	..	..	200	0.11*	1
	...	..	..	300	0.46*	1
	...	..	..	500	0.42*	1

[a] Blood samples were taken 5 to 9 minutes after intravenous injection of estradiol.
* Value significantly greater than zero ($P<0.01$).

after injection and the estrogen concentration was maintained at levels which were easily assayable. However, the rate of disappearance of the sodium salt from the blood was also quite rapid. The greatest recovery of the sodium salt in blood was obtained 9 minutes after injection. Assuming that the blood volume comprises 8% of the body weight, the maximum recovery at this time interval (table 1) indicates that only 8.6% of the injected dose was still present in the blood.

The Excretion of Estrogenic Activity in Ewes' Urine. The urinary excretion of estrogens after the intravenous injection of 1.0 mg. of microcrystalline suspension and the sodium salt of estradiol was measured in two anestrous ewes. The pattern of estrogen excretion was similar in both, but the ewe receiving the microcrystalline suspension excreted twice as much estrogen during the collection period as did the ewe receiving the sodium salt of estradiol (figure 2).

During the first 45 minutes after the exogenous estrogen was given, the highest rates of estrogen excretion were found. It was expected that the ewe receiving the sodium salt would have the greatest rate of excretion, but this was not the case. Instead, for this initial period the ewe receiving the crystalline estradiol had approximately twice the rate of excretion as that of the ewe receiving the sodium salt. This apparent deviation from the expected might be attributed to a very rapid metabolism of the sodium salt of estradiol to inactive excretion products. The kidney may also trap crystals of the free compound in the glomeruli from where estradiol molecules could be mobilized directly into the filtering system of the kidney.

During the second phase of estrogen excretion, lasting from 45 minutes to approximately 6 hours after injection of the hormone, the rate of estrogen excretion was significantly greater in the ewe receiving the crystalline suspension. This was the pattern expected during the later stages of excretion, assuming that the estradiol crystals were being held in a tissue depot site and slowly mobilized into the circulation and through the kidneys.

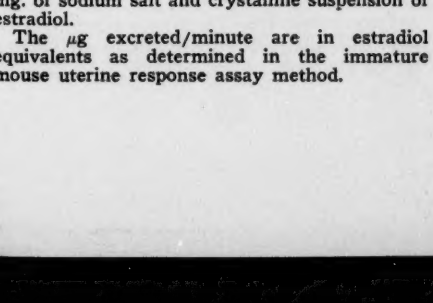

Figure 2. The urinary excretion of estrogens in two ewes after intravenous injections of one mg. of sodium salt and crystalline suspension of estradiol.

The μg excreted/minute are in estradiol equivalents as determined in the immature mouse uterine response assay method.

Summary

No assayable quantities of estrogen from an endogenous source were found in 100-ml. samples of ewes' blood. When a microcrystalline suspension of estradiol was injected intravenously in relatively large quantities, none was found in the blood. It was suggested that the microcrystals had been phagocytized and thus removed from the circulation within 5 minutes or less. The sodium salt of estradiol, when injected intravenously, was readily extractable from 100-ml. samples of ewes' blood if it were first partially hydrolyzed in an NaOH solution. This indicated that a large percentage of the estradiol was bound very rapidly to the blood proteins. Both the sodium salt of estradiol and the microcrystalline suspension when added intravenously, had similar excretion patterns, with the microcrystalline suspension appearing in the urine at twice the excretion rate of the sodium salt of estradiol and remaining at higher rates throughout the period of collection.

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ZINC REQUIREMENT OF THE GROWING PIG FED ISOLATED SOYBEAN PROTEIN SEMI-PURIFIED RATIONS

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ZINC deficiency has been demonstrated in rats on diets estimated to contain not more than 1.6 p.p.m. zinc (Hove *et al.*, 1937) and in mice on diets containing as little as 0.3 p.p.m. (Day and Skidmore, 1947). O'Dell *et al.*, (1958) produced a zinc deficiency in chicks using a diet containing 15 p.p.m. of zinc. Hogan and Johnson (1941) were the first to publish an accurate clinical description of parakeratosis. It was not until 1953 that Kernkamp and Ferrin (1953) characterized the dermatosis in swine. Tucker and Salmon (1955) reported that parakeratosis in swine was prevented or cured by the addition of 112 p.p.m. of zinc to the diet. They also showed that excesses of calcium or phosphorus or both markedly increased the severity of the disease. These results were later confirmed by Lewis *et al.* (1956), Luecke *et al.* (1956) and Conrad and Beeson (1957). Both corn-soybean oil meal and purified rations were used in those studies, the basal rations containing approximately 28 p.p.m. of zinc and from 0.7 to 1.5% calcium. In a subsequent study by Lewis *et al.* (1957) a corn-soybean oil meal type ration which contained 28 p.p.m. of zinc and 0.5% calcium did not prevent the appearance of skin lesions of parakeratosis. Using a similar ration containing 28 p.p.m. of zinc and 1.2% calcium, Luecke and Hoefer (1958) reported that the addition of 40 p.p.m. of zinc was necessary to prevent all symptoms of parakeratosis. A preliminary quantitative study by Smith *et al.* (1958) indicated that swine fed purified rations containing isolated soybean protein required at least 46 p.p.m. zinc for maximum performance. The present study was designed to characterize a true zinc deficiency and to determine the zinc requirement of growing swine.

Experimental Procedure

Three experiments were conducted, most of the experimental methods and procedures being the same for all. When the pigs were about three weeks old and weighed from 10 to 14 lb., they were removed from the sows and confined to wooden pens with concrete floors. There were two pigs per pen and six pigs per treatment. No bedding was used. Tap water was used to wash the pens. The drinking water was conveyed through a zeolite water softener. The pigs were allowed to eat *ad libitum*. They were weighed weekly, and the feed consumption was calculated each week. The basal ration, which contained 16 p.p.m. zinc was the same in all three experiments (table 1).

Experiment I. Forty-two, three-week-old pigs were divided into seven lots on the basis of weight, sex and litter. Lot 1 received the basal ration. Zinc oxide was added to the basal to provide 21, 26, 31, 36, 41 and 46 p.p.m. of total zinc in the rations of Lots 2 through 7, respectively. The depletion phase was terminated after 56 days and the pigs receiving 16, 21, 26 and 31 p.p.m. of zinc were reallotted into six lots on the basis of previous treatment and average daily gain. The following treatments, basal ration plus 50 p.p.m. zinc; thiamine, 12 mg., per lb. of ration; citric acid, 1% of ration; benadryl, 12.5 mg. per lb.; sodium formate and acetic acid (1:1 ratio), 1% of ration; and, calcium acetate and acetic acid (1:1 ratio), 1% of ration were applied to observe their effect on the apparent zinc deficiency syndrome.

Experiment II. Thirty-six, three-week-old pigs were allotted into six lots on the basis of weight, sex and litter. Zinc oxide was added to the basal ration to provide 36, 41, 46, 51, 56 and 61 p.p.m. total zinc for lots 1 through 6, respectively. The feeding period was for 63 days.

Experiment III. Thirty-six, three-week-old pigs were divided into six lots of six pigs per lot on the basis of weight, sex and litter. The

[1] The authors are grateful to Calcium Carbonate Co., Quincy, Illinois for the calcium, phosphorus and zinc analysis reported herein.

[2] Contribution from the Department of Animal Science, Journal Paper No. 1631.

TABLE 1. THE COMPOSITION OF THE SEMI-PURIFIED BASAL RATION. EXPERIMENTS I, II, AND III

Ingredient	Percent
Isolated soybean protein	25.00
Cerelose	30.88
Corn starch	30.88
Corn oil (Mazola)	5.00
Celluflour	3.00
DL-methionine	0.25
Minerals (major mix) [a]	4.00
Trace mineral mixture [b]	0.43
Vitamin—antibiotic mixture [c, d]	0.56
Choline chloride (liquid, 70% choline)	0.0017
Protein, percent of ration	22.50
Calcium, percent of ration	0.67
Phosphorus, percent of ration	0.47
Zinc, p.p.m.	16.00

[a] The following minerals of U.S.P. grade were added as percent of the ration; iodized salt, 0.5; $CaHPO_4 \cdot H_2O$, 2.0; $CaCO_3$, 0.5; KCl, 0.5; KH_2PO_4, 0.5.

[b] Trace minerals of U.S.P. Grades added per pound of ration: $MnSO_4 \cdot H_2O$, 18.0 mg.; $CoCO_3$, 0.2 mg.; CaF_2, 4.8 mg.; $CuSO_4$, 3.0 mg.; $(MgCO_3)_4Mg(OH)_2 \cdot 5H_2O$, 182 mg.; $FeSO_4 \cdot 2H_2O$, 32.6 mg.

[c] Vitamins added per pound of ration: Thiamin HCl, 3 mg.; riboflavin, 4 mg.; niacin, 15 mg.; calcium pantothenate, 10 mg.; pyridoxine, 1.5 mg.; para-amino benzoic acid, 4 mg.; biotin, 0.1 mg.; inositol, 50 mg.; folic acid, 2 mg.; ascorbic acid, 50 mg.; 2 methyl-1, 4, naphthoquinone, 1 mg.; B_{12}, 20 mcg.; D_2, 200 I.U.; D_3, 200 I.U.; vitamin A, 2000 I.U.; mixed tocopherols, 15 mg.

[d] Chlortetracycline HCl, 25 mg. per pound of total feed.

following levels of zinc: 26, 31, 36, 41, 46 and 51 p.p.m. were fed to lots 1 through 6, respectively, by adding zinc oxide to the basal ration. The experiment consisted of two phases, depletion and repletion. At the end of the 56-day depletion phase, all pigs receiving 26, 31, and 36 p.p.m. zinc were reallotted into three groups on the basis of previous treatment and sex.

The repletion treatments for groups 1, 2 and 3, respectively, were: basal plus 36 p.p.m. zinc, basal plus 10 p.p.m. zinc, and casein basal plus 10 p.p.m. zinc (commercial casein, 85% protein, was substituted for the soybean protein, 82% protein, on an equal weight basis). Thus, rations for groups 1, 2 and 3 contained 52, 26 and 28 p.p.m. total zinc, respectively. At the end of 2 weeks, pigs in group 2 were doing poorly, and casein was substituted for the soybean protein and the added zinc. The casein basal contained 18 p.p.m. zinc by analysis.

Results and Discussion

Experiment I. Table 2 summarizes the growth, feed and parakeratosis data for experiment I. The average daily gains for the 56 days were 0.06, 0.13, 0.25, 0.52, 0.61, 0.69 and 0.76 lb. for the treatments 16, 21, 26, 31, 36, 41 and 46 p.p.m. zinc, respectively. Analysis of the data indicated that the average daily gain was a linear function of the amount of zinc in the ration (Snedecor, 1953). All pigs fed 16, 21, and 26 p.p.m. zinc and four fed 31 and three fed 36 p.p.m. developed parakeratosis. The pigs receiving 41 p.p.m. zinc appeared normal. However, the pigs fed 46 p.p.m. zinc gained 9% faster than those fed 41 p.p.m. Similar results have been reported by Tucker and Salmon (1955) and Luecke *et al.* (1956) when natural rations were fed containing from 29 to 44 p.p.m. zinc. However, Luecke and Hoefer (1958) showed that 68 p.p.m. zinc were required to obtain maximum growth and prevent parakeratosis when a corn-soybean protein ration was fed.

The zinc deficiency syndrome is characterized by decreased feed and water intake, retardation in growth, scours, parakeratosis,

TABLE 2. SUMMARY OF GROWTH, FEED CONSUMPTION, FEED CONVERSION, AND INCIDENCE OF PARAKERATOSIS. EXPERIMENT I

(FEBRUARY 10 TO APRIL 10, 1958—56 DAYS)

Item	Treatment						
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6	Lot 7
No. of pigs	6	6	6	6	6	6	6
Added zinc, p.p.m.	0	5	10	15	20	25	30
Total zinc, p.p.m.	16	21	26	31	36	41	46
Average initial wt., lb.	11.69	10.92	10.71	10.88	11.00	10.00	10.96
Average final weight, lb.	15.00	18.00	24.92	40.25	44.90	49.90	53.50
Average daily gain, lb.[a]	0.06	0.13	0.25	0.52	0.61	0.69	0.76
Average daily feed, lb.	0.91	0.82	1.04	1.28	1.40	1.54	1.59
Feed per pound of gain, lb.	15.32	6.52	4.09	2.44	2.31	2.22	2.08
Parakeratosis, no. of pigs	6	6	6	4	3	0	0
Deaths	2[b]	0	0	0	1[c]	1[d]	0

[a] Analysis of the data indicated that the average daily gain was a linear function of the amount of zinc in the ration.
[b] Both deaths resulted from a zinc deficiency—one on the 54th and one on the 55th day of the experiment.
[c] Died on the 30th day of the experiment from edema disease.
[d] Died on the 31st day of the experiment from edema disease.

Figure 1. Parakeratosis in a 9-week-old pig. The pig received isolated soybean protein ration for 6 weeks which contained 16 p.p.m. zinc.

rough hair coat, slight fading in color of hair coat, loss of hair, listlessness, a stance with all four feet together and eventually death. Figure 1 illustrates the visual symptoms. The lower the amount of the zinc in the ration, the more severe were the zinc deficiency symptoms. Two of the six pigs ingesting 16 p.p.m. zinc died during the eighth week of the experiment. Feed required per pound of gain decreased from 15.32 to 2.08 lb. with each increment of zinc.

Table 3 summarizes the data for growth, feed consumption and mortality for the zinc deficient pigs fed the different compounds during the repletion period. The pigs receiving 50 p.p.m. added zinc showed marked improvement in their appetite within two days. Growth increased rapidly during the first week. Also, the skin showed marked sloughing and healing within 10 days. By the end of 28 days, the pigs had almost completely recovered. Growth, feed efficiency, or general appearance was not improved by the other treatments.

Experiment II. The growth, feed consumption, feed conversion and parakeratosis data are presented in table 4. Zinc, 41 p.p.m., gave the highest average daily gain of 1.28 lb. in contrast to the lowest of 1.21 lb. in pigs fed either 56 or 61 p.p.m. An analysis of variance of the growth data indicated no significant difference between treatments. It was difficult to explain the results of this experiment because the pigs fed 36 p.p.m. zinc revealed no signs of a zinc deficiency; whereas, in experiment I, the pigs receiving 36 p.p.m. zinc showed a retardation of growth and symptoms of parakeratosis.

The feed required per pound of gain was essentially the same for all treatments with one exception of a slightly higher feed effiicency in lot 1 (36 p.p.m. zinc). Parakeratosis was not observed in pigs on any of the treatments.

Experiment III. Growth, feed consumption, feed conversion and parakeratosis data are

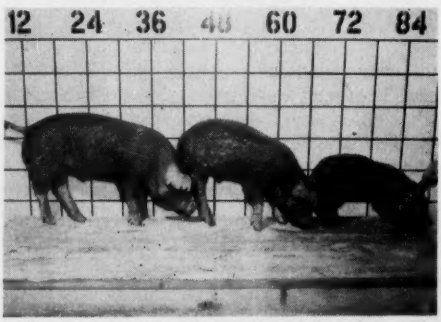

Figure 2. Levels of zinc in swine rations. Pig on the left received 46 p.p.m. zinc and appeared normal. Center pig received 36 p.p.m. zinc and exhibited moderate zinc deficiency symptoms. Pig on the right received 26 p.p.m. zinc and developed severe zinc deficiency symptoms.

TABLE 3. PERFORMANCE OF PARAKERATOTIC PIGS FED VARIOUS COMPOUNDS. EXPERIMENT I

Treatment	Item				
	Days on test	No. of pigs	Average daily gain, lb.	Average daily feed, lb.	Deaths
Basal ration plus 50 p.p.m. zinc	28	4	1.18	2.68	0
Basal plus thiamine, 12 mg. per lb.	14	4	—0.02	2.00	2
Basal plus citric acid, 1% of ration	21	4	—0.04	0.91	2
Basal plus benadryl, 12.5 mg. per lb.	21	4	—0.08	1.32	3
Basal plus sodium formate and acetic acid, 1% of ration	14	3	—0.12	1.04	1
Basal plus calcium acetate and acetic acid, 1% of ration	7	3	—0.32	1.21	1

TABLE 4. SUMMARY OF GROWTH, FEED CONSUMPTION, FEED CONVERSION, AND INCIDENCE OF PARAKERATOSIS. EXPERIMENT II
(JULY 4 TO SEPTEMBER 5, 1958—63 DAYS)

Item	Treatment					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
No. of pigs	6[a]	6	6	6	6[b]	6
Added zinc, p.p.m.	20	25	30	35	40	45
Total zinc, p.p.m.	36	41	46	51	56	61
Average initial wt., lb.	12.50	12.58	12.50	12.54	12.55	12.54
Average final wt., lb.	90.00	92.92	91.25	91.42	88.50	88.50
Average daily gain, lb.	1.23	1.28	1.25	1.25	1.21	1.21
Average daily feed, lb.	2.62	2.92	2.90	2.95	2.87	2.86
Feed per pound of gain, lb.	2.13	2.29	2.32	2.35	2.37	2.37
Parakeratosis, no. of pigs	0	0	0	0	0	0

[a] One pig was removed from the experiment on the 38th day because of edema disease, and one pig was removed from the experiment on the 62nd day because of a leg injury.
[b] One pig was removed from the experiment on the 62nd day because of edema disease.

summarized in table 5. Each increment of zinc from 26 through 46 p.p.m., increased growth. However, a very slight increase in growth of 0.04 lb. was obtained when the zinc was increased from 41 to 46 p.p.m. Either 1.00, 1.06 or 1.04 lb. average daily gain for the pigs fed 41, 46, or 51 p.p.m. zinc was significantly greater ($P<.01$) than the gain of 0.58 lb. in lot 2 (31 p.p.m. zinc).

All pigs on 26 p.p.m. zinc four pigs on 31 p.p.m. and two pigs on 36 exhibited symptoms of parakeratosis. The lower the zinc content of the ration the greater was the severity of the symptoms. Figure 2 illustrates the effect of different levels of zinc. The first apparent difference in growth was observed during the third week of the experiment. Also, one pig fed 26 p.p.m. zinc exhibited definite parakeratotic lesions during the third week on experiment.

The results of experiment III agree with those of the first experiment in that 41 p.p.m. of zinc prevented parakeratosis and 46 p.p.m. produced maximum gains.

Table 6 presents the data on growth, feed consumption and mortality during the repletion period. Marked improvement in growth, feed efficiency and general appearance was apparent for all treatments except in the group fed isolated soybean protein plus 10 p.p.m. zinc. The pigs fed casein plus 10 p.p.m. zinc (total of 28 p.p.m. zinc) consumed more feed and made an average daily gain of 2.08 lb. for the period in contrast to the group fed isolated soybean protein plus 35 p.p.m. zinc (total of 51 p.p.m.) which gained 1.37 lb. daily. Pigs receiving the casein ration (total of 18 p.p.m. zinc) gained 1.76 lb. daily. Thus, the repletion data indicated that the source of protein affected the zinc requirement of the pigs. Norris *et al.* (1957) quoted the zinc requirement for chicks under standard conditions to be not more than 20–25 p.p.m. on an isolated soybean protein-cerelose diet. Moeller

TABLE 5. SUMMARY OF GROWTH, FEED CONSUMPTION, FEED CONVERSION, AND INCIDENCE OF PARAKERATOSIS. EXPERIMENT III
(SEPTEMBER 23 TO NOVEMBER 18, 1958—56 DAYS)

Item	Treatment					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
No. of pigs	6	6	6	6	6	6
Added zinc, p.p.m.	10	15	20	25	30	35
Total zinc, p.p.m.	26	31	36	41	46	51
Average initial wt., lb.	14.10	14.35	14.25	14.38	14.33	14.29
Average final wt., lb.	30.40	47.10	61.80	70.25	73.92	72.50
Average daily gain, lb.[a]	0.29	0.58	0.85	1.00	1.06	1.04
Average daily feed, lb.	1.18	1.60	1.81	2.05	2.21	2.16
Feed per pound of gain, lb.	4.04	2.74	2.13	2.05	2.08	2.08
Parakeratosis, no. of pigs	6	4	2	0	0	0
Deaths	1[b]	1[c]	1[c]	0	0	0

[a] L.S.D. at $P<0.05=0.20$; and at $P<0.01=0.27$.
[b] The pig died during the eighth week, cause unknown.
[c] The pigs died during the sixth week, cause unknown.

TABLE 6. RESULTS OF ZINC DEFICIENT PIGS FED EITHER SOYBEAN PROTEIN OR CASEIN RATION WITH OR WITHOUT ADDED ZINC. EXPERIMENT III

Treatment	Item				
	Days on test	No. of pigs	Daily gain, lb.	Daily feed, lb.	Deaths
Isolated soybean basal ration plus 35 p.p.m. zinc	35	3	1.37	3.16	1[a]
Isolated soybean basal ration plus 10 p.p.m. zinc	14	6	—0.06	1.51	0
Casein basal ration	21	6	1.76	3.58	0
Casein basal ration plus 10 p.p.m. zinc	28	6	2.08	4.55	0

[a] The cause of death was diagnosed as a zinc deficiency.

and Scott (1958) showed essentially the same zinc requirement; however, when a cereloseegg white diet was fed, maximum growth was obtained with 10 p.p.m. of zinc. O'Dell and Savage (1957) reported that the zinc in an isolated soybean protein diet was not as available to poultry as the zinc in casein protein diets. Zeighs *et al.* (1958) confirmed these results.

Summary

Three experiments were conducted with 114 three-week-old pigs to determine the zinc requirement of the growing pig when isolated soybean protein was fed in the ration and to characterize the zinc deficiency syndrome.

The results show that zinc was a necessary constituent of the ration of the growing pig. The dietary zinc requirement of the growing pig fed semi-purified rations containing isolated soybean protein was 46 p.p.m. of the total ration in two of the three experiments. Pigs fed a ration deficient in zinc showed the following symptoms in order of appearance: decreased feed and water intake, retarded growth, scours, parakeratosis, rough hair coat, slight fading in color of hair coat, loss of hair, listlessness, a stance with all four feet together and eventually death. The parakeratotic lesions first appeared on the underneath side around the sheath and between the front and rear legs and spread to all parts of the body as the deficiency became more acute.

The dietary zinc requirement of the pig was lower when casein was substituted pound-for-pound for isolated soybean protein.

Addition of 50 p.p.m. of zinc as zinc oxide resulted in marked improvement of all zinc deficiency conditions; additions of citric acid, thiamine, benadryl, sodium formate-acetic acid mixture, or a mixture of calcium acetate and acetic acid were without effect.

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ESTROGENIC ACTIVITY OF FRESH AND DRIED RED AND SUBTERRANEAN CLOVERS [1]

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FORAGE legumes have been shown to contain compounds such as coumestrol, genistein, biochanin A, formononetin, and daidzein which produce effects on the reproductive system comparable with those induced by natural animal estrogens and synthetic estrogens such as diethylstilbestrol.

However, until more data are available on their levels in the various forages, as well as the effect of other constituents upon them, the significance of these estrogen-like compounds in livestock production cannot be properly evaluated. Additional information is needed on the stability of these compounds in forages during drying and processing.

A previous report from this laboratory has shown that estrogenic activity in dehydrated and sun-cured forage meals is highly variable (Bickoff *et al.*, 1960a). More recently, we have described the effect of forced-draft oven drying on the estrogen content of alfalfa (Bickoff *et al.*, 1960b) and white clover (Bickoff *et al.*, 1960c), as related to the growth stage at the time of cutting. The present study describes the estrogenic content of subterranean and red clover, cut at various stages of growth during a 6-month period. The effect of oven drying upon estrogenic activity is also studied.

Materials and Methods

Plantings of three varieties of subterranean clover were made at Albany, California, on June 18, 1958. Plots, each 40 ft. × 20 ft., were seeded with Dwalganup, Mt. Barker, and Tallarook varieties. At the same time, a similar planting was made from a commercial sample of red clover seed. From each of the four clovers, five sequential samples were harvested monthly, beginning with material from the first two months' growth.

For each harvest, the aerial parts from about 50 to 60 sq. ft. of the clover stand were taken. The clover harvested for assay was, as far as could be judged, representative of the entire plot. Leaves and flowers were clipped at a height of approximately one inch from the ground.

Details of handling fresh and dried forage samples were similar to those described earlier for alfalfa and white clover. They are recapitulated briefly as follows: Freshly harvested forage was rapidly macerated in a mechanical blender with acetone to extract the estrogens. Dried samples were Soxhlet extracted with acetone for 24 hours. An aliquot of each acetone extract was incorporated into the basal diet and the solvent removed. The test diets were fed to immature female mice for a period of 5 days, and the weights of freshly excised uteri were determined. The estrogenic activities were expressed in terms of the mean uterine weight for a definite weight of sample. Uterine weight on control diets averaged about 10 mg. The test diets were fed to groups of five mice per cage. In most cases, three or more replications of each sample were run at several levels. Since the curves of response to graded

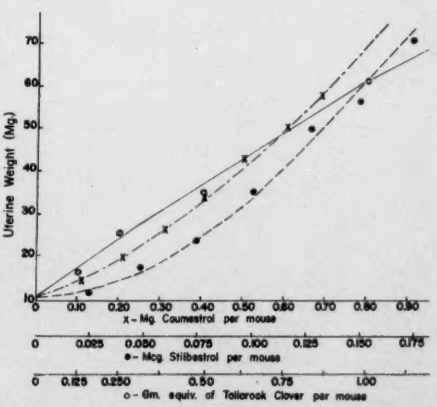

Figure 1. Uterine response to graded levels of coumestrol, diethylstilbestrol, and Tallarook clover. Each point represents the mean uterine weight value of 15 to 20 animals.

[1] The authors wish to thank Mrs. Ann R. Gramps for the preparation of many of the samples for analysis.

[2] A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

TABLE 1. ESTROGENIC ACTIVITY OF SUBTERRANEAN CLOVER OF DIFFERENT AGES

Variety	Age of stand months	Equivalent level of clover fed per mouse: Fresh samples (grams) ¼	½	1	2	4	8	Dried samples (grams) ¼	½	1	2	4	8
		Mean uterine weight responses (mg.) [a]											
Dwalganup	2	..	..	..	..	19	17	..	..	..	..	..	..
Mt. Barker		..	..	..	..	..	27	..	..	..	..	..	..
Tallarook		..	..	..	..	..	27	..	..	..	..	..	..
Dwalganup	3	..	..	..	15	37	..	..	14	38	68	71	59
Mt. Barker		..	21	47	39	..	..	..	20	48	55	..	..
Tallarook		25	35	62	72	..	..	15	21	65	46	48	..
Dwalganup	4	..	..	22	50	65	..	..	21	29	55	74	..
Mt. Barker		..	..	21	50	..	..	15	32	38	53	..	46
Tallarook		14	21	28	49	..	..	16	22	35	69	..	..
Dwalganup	5	..	18	20	47	65	..	..	..	..	..	..	..
Mt. Barker		..	16	27	56	..	..	20	45	43	53	..	..
Tallarook		..	18	32	57	..	..	..	16	45	65	60	..
Dwalganup	6	..	..	17	26	54	..	..	..	..	..	..	..
Mt. Barker		..	16	29	54	..	..	15	42	42	54	..	..
Tallarook		..	22	39	55	..	..	16	35	66	53	..	..

[a] Mean uterine weight for the control diet was 10 mg.

levels of forage extract did not have the same shape as those of diethylstilbestrol or coumestrol (figure 1), no attempt was made to express the estrogenic potencies in terms of either of these compounds.

Results

Subterranean Clovers: Two months after planting, each of the three varieties was growing well although no flowers or runners had appeared. The clover plants were about 6 inches in height on single stalks; no lateral spreading had occurred. Because of the small amount of plant material available at this time, estrogen determinations were made only on fresh material. Under the conditions of our assay, acetone extract from forage equivalent to about 8 gm. of dry weight of material of the two-month-old stand was required to produce between a two- and three-fold increase in uterine weight (table 1). The activity of this two-month-old growth was considerably less than that of any of the later samples.

At the end of 3 months, all samples exhibited lush growth. Tallarook was the most estrogenic of the three. Mt. Barker was about half as active as Tallarook, and Dwalganup, although least active, produced a significant uterine response even when fed at a level equivalent to 2 gm. of dry weight of forage per mouse.

At the end of 4 months, the Dwalganup variety had increased in activity, whereas the Mt. Barker and Tallarook had decreased from their peak activity. As a result, the estrogenic potencies of all three varieties were now quite similar. During the next 2 months, the relative potencies of Mt. Barker and Tallarook remained similar, and that of Dwalganup decreased slightly. This may be due to the fact that the Dwalganup variety was not in best condition at this time, a large number of leaves having wilted and dried.

Red Clover: No flowering occurred until the fifth harvest at the end of 5 months after planting. However, the plants grew well from the start and showed lush growth at the end of 2 months after planting when the first sampling was made and definite estrogenic activity was found (table 2). Red clover

TABLE 2. ESTROGENIC ACTIVITY OF RED CLOVER OF DIFFERENT AGES

Age of stand in months	Equivalent level of clover fed per mouse: Fresh samples (grams) 2	4	8	Dried samples (grams) 2	4	8
	Mean uterine weights (mg.) [a]					
2	24	44	55	20	25	47
3	18	30	..	17	30	..
4	19	22	40	22	44	56
5	25	50	..	19	41	..
6	..	24	58	18	28	..

[a] Mean uterine weight for the control diet was 10 mg.

maintained a relatively high activity throughout the growing period of this study although it did tend to fluctuate somewhat. Peak activity occurred at two times, namely 2 and 5 months after planting. Activity was lower after 6 months as well as during the interval between these peaks.

Discussion

Kitts *et al.* (1959) found that the estrogenic activity of red clover decreased with successive cuttings. In addition, they observed a considerable amount of activity throughout the season. Bassett and White (1955) found that the activity of red clover varied only slightly over a three-month period. In the present study, we found that total activity of both red and subterranean clover increased with age of the stand, and that there was always a significant amount of activity during the entire 6 months of the study. Neither the red nor subclovers varied widely from one cutting to the next, and after 4 months, the subclover strains seemed about equally active.

As pointed out a number of years ago (Alexander and Watson, 1951) the bioassay of a raw material such as clover is attended by uncertainties as to the number of active substances present and the augmentary or inhibitory effects of other materials also present. Since the relative proportions of these compounds may vary during the season in one plant as well as among strains or species, the forage extracts may often give different dose-response curves than do the natural or synthetic estrogens. Nevertheless, it is valuable to attempt to relate our data to some of the other figures given in the literature. This can be accomplished by relating the approximate amount of estrogenic activity in a forage to an amount of stilbestrol or estradiol. Several previous studies have been made in this country on the estrogen content of red clover. In Iowa (Cheng *et al.*, 1953) and in Indiana (Pieterse and Andrews, 1956) it was found that red clover contained the equivalent of 2 and 10 μg respectively, of stilbestrol per pound of clover. At least three reports have been made on the estrogen content of red clover grown in England. Bartlett *et al.* (1948), Legg *et al.* (1950), and Pope *et al.* (1959) have reported estrogenic activities in red clover ranging from 8 to 18 μg of estradiol per pound of dry forage.

Kitts *et al.* (1959) found that a sample of the second year growth of red clover, grown in British Columbia, had an activity as high as 36 μg of stilbestrol per pound of forage. A number of studies have been made in which the estrogenic potency of Australian and New Zealand subterranean clover was correlated with natural animal estrogens (Curnow *et al.*, 1948; Robinson *et al.*, 1949; Bassett and White, 1955). In these studies the estrogenic potency of samples of subterranean clover varied from an equivalent of 3 to 12 μg of estradiol per pound of hay. The most potent sample of subterranean clover previously found was reported by East (1950), who found a potency of 45 μg of stilbestrol per pound of dry hay.

From the results of our study, it is readily evident that some of the subterranean clover samples included in this study are unusually potent. For instant, it can be approximately estimated from the dose-response curve of stilbestrol that the three-month sample of fresh Tallarook subterranean clover had a potency equivalent to about 200 μg of stilbestrol per pound of forage. This is much higher than most of the data in the literature and certainly higher than any of the alfalfa or white clover samples that we have reported on earlier (Bickoff *et al.*, 1960b; Bickoff *et al.*, 1960c).

In spite of the relatively high estrogenic activity detected in some plant materials, inferences from these data obtained with mice as experimental animals could be misleading. It is well known that animal species differ markedly in their responsiveness to the same biological preparation. Changes to the plant estrogen that might occur in the digestive tract of animals, especially in ruminants, might result in the animal being exposed to a more potent estrogenic material than uterine weight assays of mice would indicate.

Effect of Drying: Earlier studies from this laboratory on fresh and dried alfalfa showed that some samples lost no estrogenic activity on drying, whereas others suffered a loss of as much as three-fourths or more of their original activity. Similar studies with ladino clover showed that only slight loss of activity occurred under our drying conditions. In our present studies, red clover showed slight if any loss on drying (table 2). In several cases the estrogenic activity of the dried subclover samples was definitely greater than that of the comparable fresh material (table 1). In the remaining cases, they were similar. In alfalfa and ladino clover, coumestrol is the predominant estrogen, whereas in red and subclover the estrogenic isoflavones predominate.

Swierstra (1958) has suggested that the greater retention of the activity of red and subclover during prolonged storage of the meal may reflect a greater stability of the isoflavones over coumestrol. Increased activity after drying of subclovers compared to alfalfa and white clover may also be due to a less stable estrogen inhibitor or a more stable estrogen potentiator (Bickoff *et al.*, 1960a). The complete explanation for the difference in stability of the estrogens in the various forages must await further study.

Summary

Red clover and three varieties of subterranean clover (Dwalganup, Tallarook, and Mt. Barker), planted in June, 1958, at Albany, California, were periodically assayed for estrogenic activity over a 6-month growing period. The potency of the subclovers reached a maximum between 3 and 4 months after planting, remaining high for the duration of the study, although there were some fluctuations with time as well as between varieties.

Although not as active as the subterranean clovers, red clover nevertheless maintained a relatively high activity throughout the growing period of this study. Peak activity was reached at 2 months and again at 5 months after planting.

Drying of the clover prior to assay resulted in little if any reduction in estrogenic activity. In fact, several of the dried forage samples contained more estrogenic activity than did the comparable fresh material.

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MAGNESIUM BALANCE STUDY IN PIGS THREE TO FIVE WEEKS OF AGE

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THE magnesium content of the various cereal and vegetable products fed to swine has been considered high enough to meet metabolic requirements for this ion (Cunha, 1957; Mayo *et al.*, 1959). Intensification of swine feeding will require each ingredient in the ration to be in concentrations adequate for maximum growth and fattening but not in excess. An excess of any one ingredient reduces the nutritional value of the ration.

The nutrient requirements for swine as published by the National Academy of Science-National Research Council includes magnesium as an essential dietary component but does not list a quantitative dietary requirement (Beeson *et al.*, 1959). Mayo *et al.* (1957) recommended a magnesium requirement of 270 ppm for optimum growth and feed utilization. A more detailed report by Mayo *et al.* (1959) used the criteria of weight gain, feed utilization, concentration of serum magnesium, and the prevention of signs of magnesium deficiency to determine the magnesium requirement in pigs. They concluded that the pig requires 400 to 500 ppm of magnesium to prevent signs of magnesium deficiency.

The present study was designed to determine the magnesium requirement of pigs by means of magnesium balance studies as well as by the methods used by Mayo *et al.* (1959).

Materials and Methods

In experiment I, six pigs, 25 days old were allotted to each of two dietary levels of magnesium. The pigs were from two litters of mixed Duroc and Chester White breeding. In experiment II, six Hampshire pigs, 21 days of age, were allotted to each of three dietary levels of magnesium. The litter, sex, and initial weight distributions were at random.

The composition of the rations used is given in table 1. The ration fed in experiment I was similar to one fed previously in this laboratory (Reber and Whitehair, 1955) and the ration fed in experiment II was a modification of the one used in experiment I. Roughage was added as alphacel. The lard content was decreased and the dextrose content increased. These modifications were made to prevent the spontaneous diarrhea seen in experiment I. The ration was mixed with an equal volume of distilled water to make a gruel in experiment I, but was fed dry in experiment II. In both experiments distilled water was offered *ad libitum*. The pigs were hand fed and the feed intake was recorded. The composition of the basic salt mixture used for both experiments is given in table 2. Magnesium was added as the carbonate in experiment I. Magnesium sulfate was used in experiment II. Three ration samples were obtained from each batch of ration mixed. Analyses of the rations used in experiment I showed 20 mg. and 47 mg. of magnesium per 100 gm.; for experiment II, 0.7, 3.0, and 22.1 mg. of magnesium per 100 gm. The magnesium intake for the balance study was calculated from these analyses.

The pigs in both experiments were weighed when placed on experiment and at the beginning and end of each balance period. The pigs were maintained in metabolism cages for the 17-day experimental period. These were designed to allow quantitative collection of feces and urine. No bedding was used. Feed was withheld the first day, but given twice daily thereafter. The experimental ration was fed for 10 days prior to the 7-day period during which the magnesium balance was determined. During the 7-day balance period all urine and feces from each pig were collected separately.

All of the feces excreted by each pig was dried at 105° C. for 48 hours, ground and mixed. Two samples of each collection were weighed accurately. The fat was extracted from these samples with a solution of equal parts of diethyl ether and Skelly B petroleum ether for 24 hours in the Goldfisch apparatus. The fat-extracted, fecal material was dried, weighed, and then ashed at 600° C. for 72

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TABLE 1. PERCENTAGE COMPOSITION OF THE RATION

Ingredient	Experiment I	Experiment II
Casein	35.0	35.0
Lard	40.0	20.0
Dextrose	9.0	25.0
Lactose	7.0	6.0
Salt mix	5.0	5.0
Vitamin mix[a]	2.5	2.5
Wheat germ oil (2 I.U. vit. E per gm.)	1.0	1.0
Cod liver oil (1000 U.S.P. vit. A +100 U.S.P. vit. D per gm.)	0.5	0.5
Alphacel[b]	0.0	5.0
	100.0	100.0

[a] The vitamin mix contained cyanocobalamin, 0.01 mg.; biotin, 0.025 mg.; folic acid, 0.13 mg.; menadione, 0.29 mg.; thiamine, 1.10 mg.; riboflavin, 1.80 mg.; pyridoxine, 2.00 mg.; para-aminobenzoic acid, 5.00 mg.; calcium pantothenate, 7.10 mg.; nicotinic acid, 10.1 mg.; inositol, 26.8 mg.; ascorbic acid, 130 mg.; and choline chloride, 260 mg., plus lactose to total weight of 5 gm.

[b] Non-nutritive cellulose. Nutritional Biochemicals Corp., Cleveland, Ohio.

hours. The ashed material was dissolved in 10% trichloroacetic acid and magnesium was determined on aliquots of the solutions.

Triplicate samples of about 10 gm. were taken from each ration sample. These samples were dried, fat-extracted, and ashed by the same procedure used for the fecal material.

The total urine collection from each pig was measured. At least 250 ml. was taken from the urine collected from each pig. The urine samples collected in experiment I were heated with just enough nitric acid to clear the solution. The solutions remained pale yellow. The intensity of this color was determined and subtracted from the intensity of the color in the sample following its development by titan yellow chelation for the determination of the magnesium concentration. The samples of urine obtained in experiment II were digested with sulfuric acid until clear. In both experiments the solutions were brought to pH 6 with sodium hydroxide. The solutions were diluted to a known volume prior to determination of the magnesium concentration on duplicate samples.

TABLE 2. BASIC SALT MIXTURE FOR EXPERIMENTS I AND II

Ingredients	Grams
Sodium fluoride	2.03
Potassium iodide	0.16
Ammonium alum	0.37
Manganese sulfate	0.80
Cupric sulfate·5 H_2O	0.31
Ferric ammonium citrate	61.12
Sodium chloride	616.80
Potassium phosphate (dibasic)	875.20
Potassium chloride	498.80
Calcium phosphate (dibasic)	262.50
Calcium lactate	1651.60
Calcium carbonate	274.40
Total	4244.09

Blood was collected from the anterior vena cava at the beginning and end of the balance period. Serum was drawn off after clotting and was stored at 5° C. until the magnesium concentration was determined. The protein and calcium of the serum samples were precipitated by adding 1 ml. of 10% trichloracetic acid and 2 ml. of 4% sodium oxalate to 1 ml. of serum. The samples were held at room temperature for 30 minutes. After centrifugation, a portion of the supernatant was removed for determination of the magnesium concentration. The calcium in the aliquots from the solutions of digested samples of feces, urine, and ration was precipitated by adding a volume of 4% ammonium oxalate equal to that of the aliquot.

The concentration of magnesium was determined by the intensity of the color produced by the titan yellow chelation of the ion as outlined by Garner (1946). An 0.1% solution of polyvinyl alcohol was substituted for gum ghatti. The method was also modified so that the dye and the polyvinyl alcohol could be added as one solution. The dye solution was made freshly for each series of determinations. Recovery studies showed that this modification resulted in increased precision. The concentration of ammonium oxalate altered the color intensity of the titan yellow. Care was taken to add the same amount of ammonium oxalate to the standards as that in the samples to be determined. All samples were read at 525 mμ on a spectrophotometer.[3]

Results and Discussion

During the 10-day prebalance period, all pigs in experiment I had a mild diarrhea for 1 or 2 days. Bacteriological examination of the fecal material and tissues of these pigs revealed no aerobic bacteria considered to be pathogenic. The diarrhea was probably of dietary origin, due possibly to the practice of feeding twice-a-day and making large daily increments. It may also have been due to the high fat and low roughage content of the diet.

[3] Coleman, Model 14. Coleman Instruments, Inc., Maywood, Illinois.

TABLE 3. SERUM MAGNESIUM, WEIGHT GAIN, AND FOOD EFFICIENCY OF PIGS FED VARIOUS DIETARY CONCENTRATIONS OF MAGNESIUM

Experiment	I		II		
Mg (mg. per 100 gm. ration)	20	47	0.7	3	22
Serum Mg (mg. per 100 ml.)					
Start of balance [a]	3.45	3.36	2.16	2.26	2.58
End of balance [b]	2.63	2.95	1.23	1.71[e]	2.82
Wt. gain (lb. per day) [c]	0.60	0.55	0.43	0.45	0.62
Food efficiency (gain/intake) [d]	1.25	1.18	0.85	0.92	1.03
No. showing signs of Mg def.	0/6	0/6	6/6	3/6	0/6

[a] Standard error of each mean was ±0.24.
[b] Standard error of each mean was ±0.18.
[c] Standard error of each mean was ±0.036.
[d] Standard error of each mean was ±0.069.
[e] Mean of 4 pigs: two deaths prior to final bleeding.

No diarrhea was seen after the food intake was managed more carefully. Abnormalities were not seen during the week prior to and throughout the collection period. No diarrhea was seen during experiment II.

The means for weight gain, feed efficiency, and the serum magnesium analyses are given in table 3. There was a highly significant ($P<0.01$) difference between responses of the pigs used in experiment I and experiment II, except weight gain ($P<0.05$). These disparities were probably due to differences in the breed of the pig and in composition of the ration. Incidence of signs of magnesium deficiency is also recorded in table 3.

Signs of magnesium deficiency were seen in all pigs fed 0.7 mg. magnesium per 100 gm. of ration. However, all pigs in this group survived. Three pigs fed the ration containing 3 mg. of magnesium per 100 gm. showed signs of magnesium deficiency. Two of these died the 17th day of the experiment. The first signs of magnesium deficiency were weakness in the pasterns and a lateral deviation of the hind limbs. The more severely affected pigs were hypersensitive to physical stimuli. Trembling of the skeletal musculature was common. The squeal was high pitched and raucous. Deaths occurred within 2 to 3 days after hypersensitivity became apparent. The signs appeared to be identical to those reported by Mayo *et al.* (1959).

In the experiments of Mayo *et al.* (1959), boars 3 weeks of age fed 33.4 mg. or less magnesium per 100 gm. of ration for 42 days showed signs of magnesium deficiency. They reported significant differences in weight gain between 3-week-old boars fed 7.0 mg. of magnesium per 100 gm. and those fed 20.2 mg. per 100 gm. or more. The pigs had been on experiment for 42 days before the differences were seen.

The pigs in experiments I and II fed 20 or 22 mg. of magnesium per 100 gm. may not have received the ration long enough to develop a magnesium deficiency sufficient to produce clinical signs. No significant difference in weight gain or feed efficiency due to the dietary concentration of magnesium occurred in the present study, but rations had been fed only 17 days. Development of signs of magnesium deficiency without a decrease in weight gain in pigs fed 0.7 and 3.0 mg. magnesium per 100 gm. in these experiments confirms the findings of Mayo *et al.* (1959) which indicated that the concentration of magnesium in the ration required to prevent signs of magnesium deficiency was greater than that required to promote optimum growth and food utilization. Therefore, growth and feed efficiency alone are inadequate criteria of the physiological state of magnesium deficiency.

There was no significant difference in the level of serum magnesium at the start of the collection period, but the concentration of magnesium in the ration did have a significant effect on the concentration of magnesium in the serum collected at the end of the experiment. A cubic equation was needed to fit the data adequately, since the correlation of serum magnesium concentration with concentration of magnesium in the ration was not linear. It rose rapidly as the dietary concentration increased to about 20 mg. per 100 gm. of ration, but there was little or no increase in serum magnesium between 20 and 47 mg. of magnesium per 100 gm. of ration. The data indicate that magnesium concentrations above 20 mg. per 100 gm. of ration are adequate to maintain a level of magnesium in the serum compatible with health.

The concentration of serum magnesium appears to be of little value as a criterion for establishing the magnesium requirement for swine since antibiotics may increase the concentration of serum magnesium without affecting its retention (Heggeness, 1959). The magnesium requirement inferred from growth data is inadequate to prevent the signs of deficiency. It is difficult to base the requirement for magnesium on the induction of signs of magnesium deficiency. Balance studies appear preferable as a means of determining the state of magnesium nutrition.

The retention of magnesium was calculated for each pig by subtracting the total excretion from the total intake of magnesium. The 7-day retention of each pig is plotted against its in-

take of magnesium in figure 1. The response was clearly linear; the slope of the regression line in figure 1, which indicates the rate of increased retention as the dietary concentration of magnesium was increased, was 0.87.

The magnesium found in the feces was predominantly that which was not absorbed since only a small portion of the magnesium intake. When the dietary intake of magnesium was very low, no magnesium was detected in the urine of some pigs, but there was magnesium in the feces.

The magnesium intake needed to maintain a balance of magnesium in the pigs would be the intake corresponding to the point on the graph in figure 1 where the line crosses the

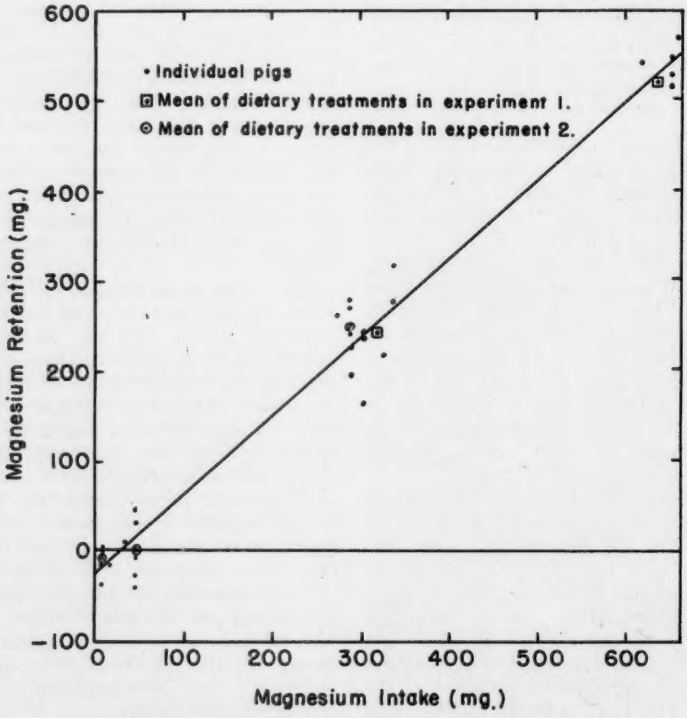

Figure 1. Magnesium intake and retention of pigs for one week.

absorbed is excreted into the gut (Elkinton, 1957; Lengemann, 1959; McCance and Widdowson, 1939). The average magnesium excreted in the feces was 65 to 84% of the total magnesium. Aikawa (1958) reported that 88% of Mg-28 given orally to human beings was recovered in the feces. The differences between the magnesium recovered in the feces and urine of the pigs might have been more distinct if some fecal magnesium had not been washed into the urine during the separation of the excreta in the metabolism cages. The quantity of magnesium in the feces and urine varied directly with the magnesium ordinate, i.e., the point where the retention of magnesium is zero. However, the growing animal needs more than just a balance of magnesium if an adequate concentration of magnesium is going to be maintained in all tissues. Shohl (1939) compiled data from the literature showing that 0.03% of the adult body and 0.024% of the newborn human is magnesium. We assume that magnesium makes up a similar amount of swine tissue. The pigs used here gained an average of 0.53 lb. or 241 gm. per day. Retention of magnesium each day would have to be 58 to 72 mg. to provide proper tissue concentra-

tion based on the assumption made. A 7-day retention on this basis would be 400 to 500 mg. of magnesium.

The magnesium concentration of the ration which provided a weekly retention of 400 to 500 mg. is between 20 and 47 mg. per 100 gm. of ration (figure 1). Assuming a straight line for interpolation between these two values, 33 mg. of magnesium per 100 gm. of ration would be required for a pig to retain 450 mg. of magnesium in a week. Estimating the magnesium requirement on the basis of the concentration of magnesium in tissue implies that pigs gaining more rapidly than those in the present experiment would need a higher concentration of magnesium in the ration. Actually, the requirement would vary with the efficiency of feed utilization. Pigs which gained more rapidly would be consuming more feed. Since the work presented here and by others (Mayo *et al.*, 1959) indicates that slightly higher levels of magnesium were not harmful for pigs, a ration concentration of 40 mg. per 100 gm. would provide a margin of safety for the differences in feed efficiency. This would provide for retention of 600 mg. of magnesium per week (figure 1).

After a much longer study of pigs 3 weeks of age at the start of the experiment, Mayo *et al.* (1959) recommended a dietary concentration of magnesium of 40 mg. per 100 gm. to prevent signs of magnesium deficiency.

The value of a balance study for determining the requirement for an ion over a relatively short time is shown in the present work. The dietary concentration of magnesium which will provide retention of magnesium adequate to maintain normal body concentration was accurately determined after a 10-day acclimatization period followed by a 7-day balance period.

Summary

Magnesium balance, weight gain, food efficiency and serum magnesium were observed in 30 pigs. Five groups each of six pigs were fed one of the following dietary concentrations of magnesium: 0.7, 3.0, 20, 22, and 47 mg. per 100 gm. of ration. Signs of magnesium deficiency which developed in pigs fed 0.7 and 3.0 mg. per 100 gm. of ration and the syndrome were described. The serum magnesium was shown to be a non-linear function of dietary magnesium concentration. Retention of magnesium over a 7-day period was shown to increase linearly with increasing magnesium intake. On the basis of balance studies, 40 mg. of magnesium per 100 gm. of ration is recommended as the dietary concentration which will maintain body concentrations of magnesium compatible with health of pigs 3 to 6 weeks of age.

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PROTEIN REQUIREMENTS OF SUCKLING AGE PIGS[1, 2]

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A RECENT development in swine production has been the early weaning of pigs. As a result of this development there is a need for information on nutritive requirements of pigs weaned at an early age.

Sows' milk contains approximately 33% of protein on a dry matter basis according to Hughes and Hart (1935), Braude *et al.* (1947), Bowland *et al.* (1949) and Heidebrecht *et al.* (1951). Reber *et al.* (1953) using vitamin-extracted casein as the sole source of protein reported that a diet containing 41% of protein produced maximum weight gains and efficiency of feed utilization of pigs from 2 to 24 days of age. As the pigs approached 8 weeks of age a 20% protein diet was as efficient as the higher protein diets. Sewell *et al.* (1953) fed crude casein and an isolated soybean protein supplemented with methionine and reported that 16 or 20% of protein produced slow growth in baby pigs from 2 to 30 days of age. Twenty-four and 28% protein diets gave relatively satisfactory results, but faster and more efficient gains were obtained by feeding a 32% protein diet.

The objective of the studies reported herein was to obtain additional information on the dietary protein requirements of pigs weaned at approximately 3 weeks of age and fed a practical-type diet.

Experimental Procedure

Two trials were conducted with a total of 39 Duroc pigs weaned at 3 weeks of age to determine the influence of protein content of the diet on weight gains and efficiency of feed utilization. The pigs were sorted into outcome groups on the basis of litter, weight and sex and allotted at random to the four treatments (16, 20, 24 and 28% of dietary protein). Soybean oil meal and tankage were included in all rations in a 2:1 ratio. The proportion of these feeds and corn was varied to obtain the specified protein levels. The composition of the rations is shown in table 1. The rations were eaten *ad libitum* and feed consumption and individual pig weights were determined at weekly intervals throughout the 5-week test period.

The feeding experiment was followed by a series of nitrogen balance experiments with 16 male pigs (barrows) fed rations of the same composition as those used in the growth studies. The pigs were weaned at approximately 3 weeks of age, weighed and placed in metabolism cages. They were then fed the ration to which they had been randomly assigned. At the completion of the first collection period each pig was assigned to another protein level so that during an experiment each of the four pigs received each of the four protein levels (16, 20, 24 and 28%). Each pig was fed the assigned experimental diet in an amount corresponding to a percentage of its bodyweight. The daily ration was determined by the pig eating the least amount of feed. Thus, during any given trial all pigs were fed an amount of feed corresponding to the same percentage of bodyweight. Once the level of feed intake was established it was maintained at this level for at least 3 days prior to collection and throughout the collection period. Four such experiments were conducted making a total of 16 nitrogen balance trials for each level of dietary protein.

Collection periods of 3 days were used during the first two experiments and of 5 days during the last two experiments. Short preliminary and collection periods were necessary in order that each pig receive each level of protein within the 3- to 9-week bracket. Lassiter *et al.* (1956) reported that after a 10-day preliminary period a 7-day collection period offered but little advantage over a 3-day collection period in the case of 50-lb.

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TABLE 1. COMPOSITION OF RATIONS USED IN GROWTH STUDIES AND NITROGEN METABOLISM STUDIES

Ration	1	2	3	4
Protein level, calculated, %	16	20	24	28
Ingredients, lb.				
Ground yellow corn	56.1	47.0	37.9	28.3
Sugar[a]	10.0	10.0	10.0	10.0
Soybean oil meal[b]	9.6	16.0	22.4	28.8
Tankage[c]	4.8	8.0	11.2	14.4
Dried skim milk	11.9	11.9	11.9	11.9
Lard-lecithin mixture[d]	5.1	5.1	5.1	5.1
Steamed bone meal	1.0	0.5	...	...
Salt (trace mineralized)	0.5	0.5	0.5	0.5
Vitamin premix[e]	1.0	1.0	1.0	1.0
Chlortetracycline	2.0 gm.	2.0 gm.	2.0 gm.	2.0 gm.
Protein, analyzed, %[f]	15.9–16.9	19.5–20.5	23.0–24.4	27.6–28.4

[a] Refined, table grade.
[b] Solvent process, 45% protein.
[c] Dry rendered, 60% protein.
[d] Approximately 80% lard and 20% lecithin.
[e] One lb. of the vitamin premix added (calculated) 4 mg. riboflavin, 8 mg. pantothenic acid, 18 mg. niacin, 20 mg. choline, 20 mcg. vitamin B-12, 4000 I.U. of vitamin A and 540 I.U. of vitamin D-2 per lb. of total feed.
[f] Represents the range of percent of protein (N x 6.25) contained in rations used during different trials. Actual values for separate rations fed during individual trials were used in calculating nitrogen intake of individual pigs.

pigs, and even less advantage with 150-lb. pigs.

A metabolism harness and collection unit as described by Kolari *et al.* (1955) was used in this study. The urine collection unit was modified during the last two metabolism experiments by placing two electrodes one-half inch apart in the floor of the collection cup. The electrodes were attached to the grid of a vacuum tube which was biased in such a manner that when the urinal was dry no plate current would flow through the tube. However, when the electrodes were covered with urine the grid of the tube was effectively grounded. This allowed plate current to flow through the tube and hence through a relay in the plate current circuit. The relay then closed, turning on the vacuum pump which withdrew the urine from the cup. A time delay relay was incorporated so that the pump would actually run for 30 seconds after the urine cup was dry. This was found to be necessary to withdraw all the liquid from the tubing before the pump stopped.

The urine volume of each pig was measured twice daily at regular intervals. A 10% sample was taken, composited with an equal volume of 5% sulfuric acid and kept at 35° F. until analyzed. Five milliliter duplicate samples were withdrawn from each composite sample and analyzed for nitrogen content.

Fecal collections were also made twice daily. The entire fecal excretion of each pig was weighed and stored at —15 to —20° F. until analyzed. The total fecal collection for each pig was mixed thoroughly and duplicate 10-gm. samples were taken for nitrogen determinations. Each 10-gm. sub-sample was digested and the digest diluted to a 250-ml. volume. Duplicate 25-ml. aliquots of each digest were then distilled in making the final nitrogen determinations. Samples of the experimental diets were taken at each feeding and a composite sample formed. The composite samples were ground through a Wiley mill fitted with a No. 70 screen. Duplicate 1-gm. samples were digested and used for nitrogen

TABLE 2. PIG WEIGHTS AND FEED REQUIRED PER LB. OF GAIN OF ANIMALS WEANED AT 3 WEEKS AND FED DIETS CONTAINING FOUR LEVELS OF PROTEIN FOR 5 WEEKS (MEANS OF TWO REPLICATES)

Treatment	1	2	3	4
Protein in the diet, %	16.9	20.5	23.0	27.6
No. of pigs per treatment	9[a]	9[a]	10	10
Av. initial weight, lb.	12.3	12.3	12.1	12.1
Av. adjusted final weight, lb.[b]	40.6	44.2	40.8	42.9
Av. feed consumed/lb. of gain, lb.	2.25	2.12	2.02	1.98

[a] Only 9 pigs used in treatment 1; one pig died in treatment 2. Calculated values were used to facilitate statistical analysis of gain data.
[b] Average final weight plus correction factors obtained by multiplying deviation from the mean initial weight (12.2) by regression coefficient (1.72) (Snedecor, 1946).

determinations. The modified macro Kjeldahl determination as used by Russo *et al.* (1954) was used in these investigations.

Results and Discussion

Growth Studies. The data presented in table 2 indicate that some differences resulted in final pig weights due to level of dietary protein. Covariance analysis of the data (Snedecor, 1946) showed that there was not a significant difference in average final weights of the pigs due to treatment. A trend toward an improvement in efficiency of feed utilization for gain is apparent with increased protein content of the diet. The pigs fed the 16.9% protein diet required approximately 11% more feed per unit of gain than the pigs fed the 23% protein diet.

If it can be assumed that the amino acid requirements of growing pigs are a linear function of the protein content of the diet when adequate but not excessive protein is present, it appears that the approximately 16% protein diet used in this experiment should have provided for satisfactory essential amino acid nutrition of pigs of the age and weight used. Becker (1959) has suggested that the requirement for the essential amino acids, expressed as a percentage of dietary protein, decreases linearly as the dietary protein increases, the rate of decrease being 1.75% (from its value at 0% protein) per unit increase in percent of dietary protein.

The calculated percentages of the essential amino acids in the "16, 20 and 24% protein diets" fed and the suggested requirements of Becker (1959) are given in table 3. The calculations of amino acid content of the rations are based on published data on feeds of similar composition to those used in these studies (Lyman *et al.*, 1956; National Research Council, 1959). It is evident from the data presented in table 3 that the 16% protein diet should have provided for satisfactory essential amino acid nutrition for growth of pigs such as those used in this study.

Crampton and Ness (1954) weaned pigs at 10 days of age and found that increasing the protein content of the diet from 26% to 30% resulted in significant increases in gain (28%) and efficiency of feed utilization (15%). Smith and Lucas (1956) reported significant improvement in feed conversion but no significant effect on growth when the protein content of the diet was increased from 24 to 29% for pigs weaned at 10 days and fed to a weight of 25 lb. In a subsequent study Smith and Lucas (1957) reported no advantage from feeding more than 18% protein to pigs during the growth period of 25 to 50 lb. They did not feed a level below 18%. Peo *et al.* (1957) reported that pigs weaned at one week of age and fed for four weeks made maximum gains when they were fed a 20% protein diet in which dried skim milk and soybean oil meal were the major sources of protein. Becker *et al.* (1954) used dried skim milk as the sole source of protein and reported that pigs from one to four weeks of age required 22% protein, whereas 12% was the minimum required by pigs from five to nine

TABLE 3. COMPARISON OF CALCULATED ESSENTIAL AMINO ACID CONTENT OF DIETS FED AND SUGGESTED REQUIREMENTS FOR THESE AMINO ACIDS [a]

Percent of protein in diet	16		20		24	
Amino acid	Req't [b]	Calculated	Req't [b]	Calculated	Req't [b]	Calculated
Arginine	0.25	0.82	0.29	1.11	0.31	1.40
Histidine	0.23	0.42	0.26	0.53	0.28	0.65
Isoleucine	0.52	0.82	0.58	1.00	0.63	1.19
Leucine	0.67	1.50	0.77	1.80	0.83	2.09
Lysine	0.74	0.92	0.84	1.22	0.90	1.51
Methionine [c]	0.50	0.29	0.56	0.34	0.60	0.39
Phenylalanine	0.54	0.76	0.61	0.95	0.65	1.14
Threonine	0.45	0.62	0.51	0.78	0.55	0.92
Tryptophan	0.12	0.18	0.14	0.23	0.15	0.28
Valine	0.46	0.95	0.52	1.20	0.56	1.45

[a] Expressed as percent of diet.
[b] Suggested requirement (Becker, 1959).
[c] Does not include cystine in calculated content of diets. Cystine can replace 40% (Becker, 1959) or 50% (N.R.C., 1959) of the methionine requirement.

TABLE 4. INFLUENCE OF LEVEL OF DIETARY PROTEIN ON NITROGEN METABOLISM

Percent of dietary protein	No. trials	Mean $W_{kg.}^{0.75}$	Average daily N metabolism					Percent ingested N absorbed	Percent ingested N retained	Percent absorbed N retained[b]
			Intake, gm.	Feces, gm.	Absorbed, gm.	Urine, gm.	Retained, gm.	Retained/ $W_{kg.}^{0.75}$, gm.[a]			
16	16	6.37	14.30	2.52	11.78	4.11	7.67	1.18	82.37	53.57	65.02
20	16	6.35	17.48	2.81	14.67	5.19	9.48	1.46	83.92	54.23	64.62
24	16	6.42	20.87	3.24	17.63	7.47	10.16	1.57	84.47	48.68	57.63
28	16	6.55	24.48	3.21	21.27	10.67	10.60	1.60	86.89	43.30	49.83

[a] Differences in amount of nitrogen retained daily per $W_{kg.}^{0.75}$ are highly significant ($P<0.01$).
[b] Highly significant ($P<0.01$) differences in percentage of absorbed nitrogen retained due to protein level.

weeks of age. A subsequent study by the Illinois workers (Jensen *et al.*, 1957) indicated that the protein requirement of pigs from two to eight weeks of age was approximately 17% when corn and casein were the sources of protein. The results of the growth studies reported herein are in general agreement with those of Peo *et al.* (1957) and Jensen *et al.* (1957). In each of these studies, efficiency of feed utilization tended to increase as protein level increased, within the range of 15 to 30%.

Nitrogen Balance Studies. The data presented in table 4 demonstrate that as the level of protein was increased from approxmately 16 to 28% of the diet there was an increase in the total nitrogen retained per day, slight increases in apparent digestibility of nitrogen, and decreases in the percentages of ingested and apparently absorbed nitrogen retained. Analysis of variance of the data,

TABLE 5. ANALYSIS OF VARIANCE OF DATA FOR PERCENTAGE OF ABSORBED NITROGEN RETAINED

Source of variation	Degrees of freedom	Mean square
Experiments	3	204.40
Pigs	12	11.88
Age (trials)	3	169.10
Protein levels—linearity	1	2421.38**
deviations	2	120.49
Experiments x age	9	89.77
Experiments x protein levels	9	41.14
Error	24	76.73

** $P<0.01$.

table 5, showed a highly significant ($P<0.05$) difference in percentage of absorbed nitrogen retained due to level of dietary protein. Likewise, a highly significant linear trend was obtained indicating that as the percentage of protein in the diet was increased the percent of apparently absorbed nitrogen retained by the pigs decreased.

The percentages of absorbed nitrogen retained by the pigs fed the 16 and 20% protein diets did not differ significantly from one another, but did differ significantly from the percentages of absorbed nitrogen retained by the pigs fed the 24 and 28% protein diets, when tested by the multiple range test (Duncan, 1955).

The significant trend toward a decreased percentage of absorbed nitrogen retained with increasing protein content of the diet indicates that when the diets contained more than 16 or 20% of protein the pigs were being fed

protein in excess of their needs. As is shown in table 4, there was a marked increase in the daily loss of nitrogen in the urine with increasing level of dietary protein. On the other hand, the pigs fed the higher protein diets retained more total nitrogen per day than did those fed the low protein diet, but the quantitative differences became markedly less as the level of dietary protein was increased.

This trend was also apparent in the average nitrogen retention values per unit of metabolic size ($W_{kg.}^{0.75}$). The mean values obtained when the pigs were fed the 20, 24 and 28% protein diets are not greatly different and do not differ significantly. However, there is a highly significant ($P<0.01$) difference in nitrogen retention per unit of metabolic size, all levels of dietary protein being considered. These results also suggest that in studies of this nature it is preferable to express nitrogen on the basis of nitrogen retention per unit of metabolic size rather than on the basis of percentage of either ingested or absorbed nitrogen retained.

The results as presented in table 4 and discussed above demonstrate that a diet containing approximately 20% of protein of high quality is required to promote satisfactory nitrogen retention. This finding differs from the growth studies in that a diet containing 16.9% of protein was adequate to promote acceptable rate of gain in 3-8-week old pigs. Thus, it appears that the amount of dietary protein required to promote good weight gains is somewhat less than the amount needed to promote satisfactory nitrogen retention in rapidly growing pigs.

Pig age and pig size also appeared to be factors influencing urinary nitrogen excretion. As the pigs became older and heavier there was a definite trend toward increased loss of nitrogen in the urine at all levels of protein. This situation indicates that diets containing 20% or more of protein were more than adequate for 3- to 8-week old pigs during the latter stages of the trials, but that a diet containing at least 20% of protein is required by pigs weaned at 3 weeks of age and during the early stages of the subsequent growth period.

Summary

Thirty-nine pigs weaned at 3-weeks of age were used to obtain further information on the protein requirement for growth of pigs during the period of 3- to 8-weeks of age. No significant difference resulted in rate of gain of the pigs due to the use of rations containing approximately 16, 20, 24 and 28% of protein. There was a definite trend toward more efficient utilization of feed for gain as the level of dietary protein increased.

Nitrogen balance studies were conducted to determine the influence of level of dietary protein upon nitrogen retention by pigs fed 16, 20, 24 and 28% protein diets during the period from 3- to 8-weeks of age. A highly significant difference ($P<0.01$) resulted in the percentage of absorbed nitrogen retained due to protein level. Likewise, there was also a highly significant difference in amount of nitrogen retained per unit of metabolic size ($W_{kg.}^{0.75}$) due to protein level with significantly less nitrogen being retained by the pigs fed the 16% protein diet than by the pigs fed diets containing any of the other levels of protein. It appears that the percentage of dietary protein required to promote acceptable gains in 3- to 8-week old pigs is somewhat less than that required to promote satisfactory nitrogen retention. It is suggested that pigs weaned at 3 weeks of age require at least 20% of dietary protein of high quality to promote maximum nitrogen retention, during the early stages of the subsequent growth period.

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INFLUENCE OF LEVEL OF DIETARY PROTEIN ON LIVE WEIGHT GAINS AND CARCASS CHARACTERISTICS OF SWINE [1]

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SINCE the advent of antibiotics and vitamin B_{12}, numerous investigators (Catron *et al.*, 1952; Hoefer *et al.*, 1952; Becker *et al.*, 1954; Hanson *et al.*, 1955; Jensen *et al.*, 1955; Meade, 1956) have demonstrated that growing-finishing pigs will gain weight rapidly and efficiently when fed rations containing less protein than previously recommended, provided the rations are adequate in non-protein dietary constituents. Some workers have also studied the influence of dietary protein level on carcass characteristics. Catron *et al.* (1952) reported that the protein content of a ration had no significant effect on the lean content of carcasses of pigs. In a more comprehensive study Ashton *et al.* (1955) found that carcass lean, as measured by specific gravity and percent lean cuts, was increased significantly as the protein level of the diet increased from 10 to 20%. The protein effect on leanness between any two adjacent protein levels was of small magnitude, however. This study differed from the work reported by Catron *et al.* (1952) in that the animals were fed the same level of protein throughout the experimental period.

The experiments reported herein were conducted to obtain additional information on the influence of protein content of the ration on the carcasses from swine fed well-balanced rations, including an antibiotic.

Experimental Procedure

Two hundred twenty-six weanling pigs were fed in four experiments during a period of 3 years. In the first three experiments protein was fed at two levels; littermate pairs of pigs were selected and one pig of each pair was assigned to each protein level. In the fourth experiment protein was fed at three levels; littermate trios were selected and one pig of a trio was assigned to each level. In each experiment the sexes were evenly distributed and initial weights were similar.

Experiment 1 included 72 pigs, 12 pairs each of purebred Duroc, Chester White and Poland Chinas, with an average initial weight of 41 lb. All of the pigs had been weaned at 3 weeks and fed a 20% protein pig starter up to 8 weeks, when they were started on experiment.

Experiment 2 included 46 pigs, 23 pairs of crossbreds (Minn. No. 1 x Minn. No. 2), with an average initial weight of 60 lb. These pigs had been weaned at 56 days and were started on the experiment at 72 days of age. They were fed a commercial 22% protein starter during the suckling period.

In Experiment 3, 48 pigs, 8 pairs of Duroc x Yorkshire, 2 pairs of Duroc x Poland China, 4 pairs Chester White x Duroc and 10 pairs of Chester White x Yorkshire, averaging 38 lb. were fed. All were weaned at approximately 3 weeks of age and fed a 20% protein starter until they were started in the experiment at 8 weeks of age.

Experiment 4 included 60 pigs. There were 10 animals of each of the Yorkshire and Duroc breeds (purebreds) per treatment. These pigs also were weaned at approximately 3 weeks and fed a 20% protein starter until they were started in the experiment at 9 weeks, weighing an average of 38 lb.

In the first three experiments the initial protein levels were approximately 17% and 15% (estimated 18 and 14%). The pigs were shifted to the lower level of protein (17 to 15% and 15 to 11%) on an individual basis when they weighed 120 to 130 lb. In the fourth experiment the initial protein levels were approximately 16%, 14% and 12%. The pigs fed the two higher levels were shifted to the 11% protein level on an individual basis

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TABLE 1. PERCENTAGE COMPOSITION OF RATIONS

Experiment no.	1, 2 and 3				4			
Feeding period	Start to 125 lb.		125 lb. to finish		Start to 125 lb.			125 lb. to finish
Estimated protein, %	18	14	15	11	16	14	12[a]	11
Ingredients[b]								
Yellow corn, ground	70.1	79.9	78.3	88.2	75.0	81.0	85.3	88.2
Soybean oil meal (44%)	14.5	8.2	9.2	2.8	11.2	7.4	4.5	2.8
Tankage (dry rend. 60%)	6.2	3.6	4.0	1.2	5.0	3.4	2.0	1.2
Linseed oil meal (36%)	3.1	1.8	2.0	0.6	2.5	1.7	1.0	0.6
Alfalfa meal (dehy. 17%)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Steamed bone meal	0.3	0.7	0.7	1.4	0.5	0.7	1.4	1.4
Salt, trace mineralized[c]	0.5	0.5	0.5	0.5	...	...	...	...
Salt (plain)	...	...	...	...	0.5	0.5	0.5	0.5
Trace mineral premix[d]	...	...	...	...	0.05	0.05	0.05	0.05
Vitamin-antibiotic premix[e]	0.3	0.3	0.3	0.3	0.25	0.25	0.25	0.25
Protein, by analyses,[f] %	17.2	14.8	15.2	10.8	16.5	14.3	12.4	10.7

[a] This mixture was fed throughout the experiment to one lot of pigs.
[b] All ingredients expressed as lb. air-dry feed.
[c] Salt guaranteed to contain 0.005% Zn; in experiment 3, 11 ppm. Zn was added to all rations.
[d] Supplied 25 ppm Zn.
[e] Supplied the following per lb. of ration: 1 mg. riboflavin, 2 mg. Ca pantothenate, 4.5 mg. niacin, 5 mg. chlortetracycline and 5 mcg. vitamin B_{12}. Approximately 6 mg. chlortetracycline was supplied in Exp. 4.
[f] Adjusted to 86% dry matter.

when they weighed 120 to 130 lb. and the third group was fed 12% protein throughout the experiment.

The composition of the rations fed is given in table 1. All mixtures included 5% dehydrated alfalfa meal. The proportions of soybean oil meal, tankage and linseed oil meal were maintained at proportions of approximately 61, 26 and 13%, respectively, and the various protein levels were obtained by adjusting the amount of these ingredients and corn.

Individual pigs were removed from test when they reached weights of 195–215 lb., most of them being in the 200–210 lb. range. Those pigs which were slaughtered in the University facilities were shrunk overnight prior to slaughter. Pigs which were slaughtered at Swift and Company were marketed through normal channels on the terminal market and were slaughtered the day following shipment. Regardless of the place of slaughter the following carcass data were obtained: carcass weight (24-hour chill), dressing percentage, length of carcass, average back fat thickness and the percentages of lean and fat tissue as determined by the method of Aunan and Winters (1952). The cross sectional area of the longissimus dorsi was determined at the 10th rib on carcasses from pigs fed in Experiments 3 and 4. The percentage yield of the four lean cuts of chilled carcass was also determined in Experiments 2, 3 and 4, for those pigs slaughtered at the University.

In those instances where all pigs from a given experiment were not slaughtered, as evidenced by the discrepancy in numbers shown in tables 2 and 3 between number of pigs started and number of pigs slaughtered, the carcass data on the littermate pig to the one not slaughtered are not included. All carcass data were statistically treated by analysis of variance (Snedecor, 1948) as were the date for rate of gain.

Results and Discussion

Carcasses. The results of Experiments 1, 2 and 3 are presented in table 2. As is evident from these data and the statistical analysis neither of the protein level feeding sequences had a significant influence on any of the measures of carcass quality. However, the data from Experiment 1 do show that breed of swine influenced some measures of carcass excellence. Analysis of variance of the data showed highly significant differences ($P<0.01$) in average back fat thickness, and in percentages of fat and lean in the carcass due to breed. The differences in these measures of carcass merit, as caused by breed, can be explained on the basis of the wide differences between the three breeds as they were represented in the Station herd at that time.

TABLE 2. INFLUENCE OF LEVEL OF DIETARY PROTEIN ON CARCASSES AND ON RATE AND EFFICIENCY OF GAIN OF GROWING-FINISHING SWINE. EXPERIMENTS 1, 2 AND 3

Experiment No.	1				1				2		3	
Protein level sequence, %	18–15				14–11				18–15	14–11	18–15	14–11
Breed	Duroc	White Chester	China Poland	All pigs	Duroc	Chester White	Poland China	All pigs	Cross-bred	Cross-bred	Cross-bred	Cross-bred
No. pigs started	12	12	12	36	12	12	12	36	23[a]	23	24	24
No. carcasses	12	11	7	30	12	11	7	30	22	22	22	22
Means												
Carcass wt., lb.	142.2	145.6	144.6	144.0	145.6	149.6	144.4	146.8	142.5	141.1	145.9	148.6
Dressing, %	69.1	71.0	71.3	70.3	69.9	70.9	70.6	70.4	69.9	70.2	72.6	72.8
Carcass length, in.	28.3	27.9	27.4	27.9	27.8	28.2	27.8	27.9	30.1	30.0	29.3	29.3
Backfat thickness, in.[d]	1.88	1.80	1.40	1.74	2.00	1.83	1.48	1.82	1.62	1.65	1.56	1.63
Fat content,[b, d] %	51.5	49.6	43.7	49.0	54.3	49.3	43.6	50.0	46.7	47.8	46.3	46.3
Lean content,[b, d] %	35.2	36.7	41.2	37.2	33.0	36.8	41.4	36.4	38.9	38.1	39.2	39.2
Four lean cuts of carcass,[c] %	....	....	....	....	....	....	....	....	45.2	44.4	51.0	49.9
Loin eye area, sq. in.	....	....	....	....	....	....	....	....	....	....	4.06	3.82
Initial live wt., lb.	43.2	36.1	44.3	41.2	42.8	35.8	44.5	41.0	60.0	60.0	38.2	38.2
Final live wt., lb.	205.8	203.3	202.7	203.9	208.3	210.9	200.4	206.5	211.3	209.2	208.4	210.2
Daily gain, lb.												
Start to 125 lb.	1.67	1.46	1.48	1.53	1.65	1.51	1.43	1.53	1.88	1.93	1.67	1.64
Start to final wt.[d]	1.73	1.56	1.55	1.62	1.81	1.71	1.52	1.68	2.01	1.99	1.77	1.75
Feed/100 lb. gain, lb.	...	...	...	400	...	...	...	382	363	375	382	382

[a] One pig died after 89 days on experiment; missing value calculated for analysis of daily gain.
[b] Estimated by core sampling technique (Aunan and Winters, 1952).
[c] Data from 10 carcasses (9 barrows, 1 gilt) per treatment in Exp. 2; data from 14 carcasses (8 barrows, 6 gilts) per treatment in Exp. 3.
[d] Highly significant differences ($P<.01$) in back fat thickness, fat content, lean content and rate of gain due to breed in Exp. 1. No significant differences in any of the experiments due to treatment.

TABLE 3. INFLUENCE OF LEVEL OF DIETARY PROTEIN ON CARCASSES AND ON RATE AND EFFICIENCY OF GAIN OF GROWING-FINISHING SWINE. EXPERIMENT 4

Protein level sequence, %	16–11			14–11			12–12		
Breed	Duroc	York-shire	All pigs	Duroc	York-shire	All pigs	Duroc	York-shire	All pigs
No. pigs started	10	10	20	10[a]	10	20	10	10	20
No. carcasses	9	8	17	9	8	17	9	8	17
Means									
Carcass wt., lb.	141.0	144.0	142.4	144.4	142.3	143.4	141.0	141.8	141.4
Dressing, %	69.0	69.6	69.3	69.4	69.6	69.5	68.8	69.0	68.9
Carcass length, in.[d]	29.0	30.5	29.7	29.0	30.0	29.5	29.4	30.0	29.7
Backfat thickness, in.[d]	1.71	1.49	1.61	1.73	1.42	1.58	1.60	1.52	1.56
Fat content,[b, d] %	49.3	44.5	47.0	49.2	42.9	46.2	45.8	44.5	45.2
Lean content,[b, d] %	37.0	41.2	39.0	37.0	41.9	39.3	39.6	40.6	40.1
Four lean cuts of carcass,[c, d] %	46.7	54.2	49.5	46.1	51.4	48.2	47.7	54.3	50.2
Loin eye area, sq. in.[d]	2.97	4.08	3.49	2.96	3.78	3.35	3.15	3.99	3.55
Initial live wt., lb.	38.3	38.9	38.6	36.4	39.9	38.2	37.9	39.2	38.6
Final live wt., lb.[a]	204.3	206.9	205.5	208.1	204.4	206.4	205.1	205.4	205.2
Daily gain, lb.									
Start to 125 lb.[e]	1.58	1.50	1.54	1.52	1.44	1.48	1.37	1.24	1.31
Start to final wt.[f]	1.68	1.53	1.60	1.70	1.44	1.57	1.56	1.31	1.44
Feed/100 lb. gain, lb.	...	...	386	...	...	386	...	...	387

[a] One pig died after 41 days on treatment; missing value calculated for analysis of daily gain. Final weights of pigs slaughtered.
[b] Estimated by core sampling technique (Aunan and Winters, 1952).
[c] Based on 8 animals per treatment; 5 Duroc and 3 Yorkshire, of which 5 were barrows.
[d] Highly significant differences ($P<0.1$) due to breed. Differences due to treatment were not significant.
[e] Highly significant differences ($P<.01$) in rate of gain from start of test to 125 lb. due to treatment; significant difference ($P<.05$) due to breed.
[f] Significant differences ($P<.05$) in rate of gain for entire feeding period due to treatment and highly significant differences ($P<.01$) due to breed.

Three protein level sequences were fed in Experiment 4 and the results of this experiment are shown in table 3. As was true in the first three experiments, level of dietary protein did not exert a significant influence on any one of the items of measurement of carcass excellence, including the percentage of four lean cuts of chilled carcass and cross sectional area of the longissimus dorsi (10th rib), neither of which was determined in Experiment 1. Highly significant differences ($P<.01$) resulted, however, due to breed with the Duroc and Yorkshire breeds being represented in this experiment. Within treatment and within experiment the Yorkshire pigs showed greater carcass length, less back fat, less carcass fat, greater carcass lean, higher yield of the four lean cuts of the chilled carcass and greater cross sectional area of the longissimus dorsi.

The failure in these experiments to obtain significant differences in carcass composition due to treatment is in agreement with the observations of Catron *et al.* (1952) made from fewer data. Wallace *et al.* (1954) compared three protein levels and did not obtain a significant effect on any carcass characteristic but dressing percentage. Their leanest pigs were produced on the highest protein level fed (20.9%, initially). The extensive studies of Ashton *et al.* (1955) revealed a significant linear effect on carcass leanness due to protein. However, the carcasses were from pigs fed dietary protein at constant levels of 10, 12, 14, 16, 18 or 20% from about 35 lb. to 200 lb. Differences between carcasses of pigs fed adjacent protein levels, within the range used in the experiments reported here, were of small magnitude. Bowland and Berg (1959) found that the protein levels they used had no statistically significant effect on carcass characteristics, but that there was a trend toward greater leanness with the higher levels. It will be noted in the data shown in tables 2 and 3 that there were consistent small differences in some measures of carcass leanness in favor of the higher protein level.

Kropf *et al.* (1959) fed 16 or 12% protein

throughout the feeding period from 43 lb. to slaughter. They found that the higher protein level produced a significant increase in the percent of four lean cuts of carcass from gilts. The difference between barrow carcasses due to protein was not significant. The measurements given for this item in table 3 are for five barrow and three gilt carcasses per treatment.

The studies of others, reviewed above, as well as the data presented herein indicate that the effects of dietary protein on carcass quality are relatively minor, unless grossly inadequate protein levels are fed. The data presented in tables 2 and 3 for Experiments 1 and 4 show that a much more important consideration is the genotype of the animals.

Daily Gains. Analysis of variance of the data for rate of gain failed to show a significant difference due to treatment within any one of the first three experiments (table 2). Highly significant ($P<0.01$) differences in rate of gain resulted due to breed in Experiment 1. Likewise, highly significant differences in rate of gain resulted between experiments. These differences are attributed to differences in genetic background and to seasonal effects and not to treatment. Generally, these results agree with those of Hoefer *et al.* (1952), Wahlstrom (1954), Hanson *et al.* (1955), Jensen *et al.* (1955) and Meade (1956) that within a protein level range as represented in these experiments there was no significant influence of protein content of the ration on rate of gain. In none of the experiments was there a large difference in efficiency of feed utilization due to level of dietary protein.

In Experiment 4, highly significant differences ($P<.01$) in rate of gain due to level of protein were observed during the period up to 125 lb. This is evidence that the 12% protein ration fed was inadequate. The difference in daily gain up to 125 lb. due to breed was also significant ($P<.05$). This breed difference increased during the period from 125–200 lb.; and analysis of the data for the entire experiment showed the difference to be highly significant ($P<.01$). The effect of initial protein level on gains decreased as the animals grew heavier and older. However, further analysis of the data showed that the protein effect for the entire experiment was significant ($P<.05$), even though the three treatment groups gained at nearly identical rates after they reached a weight of 125 lb.

Summary

Two hundred twenty-six weanling pigs were fed in four experiments to determine the influence of protein content of otherwise well-balanced rations on various measures of carcass merit and on rate of gain and efficiency of feed utilization.

Protein levels compared in three experiments were 18 and 14% initially, each level being reduced three percentage units when the pigs weighed 125 lb. In the fourth experiment initial levels of 16 and 14%, reduced to 11% at 125 lb. were compared with a 12% level fed throughout the experimental period.

Carcass measurements included average back fat thickness, length, percent lean and fat and dressing percent. In all but the first experiment the percent of four lean cuts of chilled carcass was measured and the loin eye area was measured in the last two experiments.

One hundred ninety-nine carcasses were evaluated. Differences due to protein level were not significant statistically. There were fairly consistent small differences in several measurements in favor of the higher protein levels. There was a highly significant effect of breed ($P<.01$) on all measures of carcass leanness and fat in each of two experiments in which purebred animals were fed.

Initial protein levels of 14, 16 or 18% did not have a significant effect on daily gains. A highly significant decrease ($P<.01$) in daily gain was observed when weanling pigs were fed a 12% protein diet up to a weight of 125 lb., as compared with those fed 14 or 16% protein. In two experiments there were highly significant differences in daily gain between breeds.

The data support the thesis that the genotype of the animal fed is the important factor in carcass leanness and that initial protein levels for weanling pigs within the range of 14 to 18% have only minor effects on carcass quality.

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THE EFFECT OF BREED OF SIRE ON BODY SIZE OF LAMBS AT BIRTH[1]

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THE production of early milk fat lambs is the most important phase of the sheep enterprise in the native lamb states of central and southeastern United States. In these states grade ewes, either native or crossbred westerns, are bred to rams of the various mutton breeds to produce a desirable market lamb. The native ewes usually are predominantly grade Hampshires. The western ewes are generally crosses of Hampshire, Suffolk, Columbia, or Corriedale rams on grade range ewes of predominantly Rambouillet or Merino breeding. The most popular breeds of rams are Hampshire, Suffolk, Southdown, Shropshire, and Dorset.

Many producers believe that certain breeds of rams sire lambs that are heavier at birth and have proportionally larger heads than certain other breeds, thus causing increased difficulty at lambing. Differences in birth weights due to breed of sire have been reported by Miller (1935); Spencer *et al.* (1942); Kincaid (1943); Kean and Henning (1949); Blackwell and Henderson (1955); and Carter *et al.* (1957). Miller (1935) and Neville *et al.* (1958) reported breed of sire differences in other body measurements.

The purpose of the present study was to investigate the effect of breed of sire on body weight, head circumference, chest circumference, and wither height of mutton type lambs at birth.

Materials

The data were obtained from records taken at birth on 1125 lambs, born between 1941 and 1951 inclusive, at the Virginia Agricultural Experiment Station. The ewes in the flock were grade native blackface of predominantly Hampshire breeding, and Hampshire × Rambouillet crossbreeds obtained from the northwest range states. The composition and management of this flock was described in detail by Kincaid and Carter (1953).

A total of 65 rams of seven breeds, 23 Hampshire, 25 Southdown, 4 Shropshire, 4 Dorset, 2 Columbia, 2 Corriedale and 5 Hampshire × Southdown crossbreeds, were used in the flock during the 11 years covered by this study. Rams of all breeds were not used in each of the years; however, Hampshires and Southdowns did appear in all years. All the rams used were purebred except the Hampshire × Southdown, which resulted from the mating of purebreds of the respective breeds. The rams were either raised by the Virginia Agricultural Experiment Station or purchased from breeders in Virginia, usually at purebred commercial ram sales. They were considered representative in type and quality of purebred rams of the various breeds available to commercial sheepmen of Virginia and adjoining states. Each year equal numbers of ewes, classified according to age and breed, were randomly assigned to each sire and maintained as separate flocks during the breeding season. After the breeding season the rams were removed from the flock and the ewes were carried as one composite flock.

The lambs were identified, and the various observations used in this study recorded within 24 hours of birth. Birth weight was recorded to the nearest 0.1 lb. Measurements of chest circumference, head circumference, and wither height were taken with a tape to the nearest 0.1 in. The circumference of the head was measured by passing a tape around the head above the eyes and under the lower jaw at the junction of the throat. Chest circumference was taken by passing a tape around the body immediately behind the forelegs, representing a transverse vertical plane. Wither height at the highest point of the withers was taken when the animal was in a normal standing position. The tape was placed snugly on or around that portion of the body

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TABLE 1. NUMBER OF LAMBS BY BREED OF SIRE AND YEAR

Breed	Year, 19–										
	41	42	43	44	45	46	47	48	49	50	51
Hampshire	52	64	63	44	39	57	32	22	19	11	24
Southdown	58	72	48	53	44	32	31	21	27	34	24
Shropshire	..	..	..	..	..	..	..	7	11	18	22
Dorset	..	..	..	..	..	..	..	25	17	16	17
Columbia	..	..	..	..	..	28	16	..	..	..	..
Corriedale	..	..	..	..	..	2	16	8	..	..	..
Hamp. × S.D.	..	..	..	..	..	..	..	..	11	13	27

to be measured. Similar methods were followed each year. All of the measurements were taken by an experienced individual; however, it was not possible for the same individual to take the measurements and record the information throughout the study. The recorded observations on wither height were more variable than the other linear measurements, as it was extremely hard to get all lambs to stand in a similar upright position. The numbers of lambs providing data for this study are shown in table 1.

Results and Discussion

Least squares estimates of the effects due to breed of sire, and sex and type of birth of the lamb, together with their standard errors, are shown in table 2. Differences due to age of dam and year of birth were small and were not included in the table. Means by breeds of sires, adjusted for type of birth, sex, age of dam, and year of birth are shown in table 3. Differences between the adjusted means for all breed of sire comparisons, together with their standard errors, are shown in table 4.

Differences in birth weight due to type of birth and sex were in close agreement with those reported by other investigators previously cited. Lambs born as singles averaged 1.88 lb. heavier than twins, and ram lambs were 0.36 lb. heavier than ewes. Similar differences due to sex and type of birth were observed in the linear body measurements.

Lambs sired by Columbia rams were heavier than those by any breed studied and were significantly heavier than lambs by Southdowns ($P<.01$) and Dorsets ($P<.05$). Hampshire sired lambs were heavier (½ to ¾ lb.) than those by Southdowns ($P<.01$), Dorsets ($P<.01$), or Shropshires ($P<.05$). Those by Hampshire × Southdown rams were significantly heavier than Southdown sired lambs ($P<.01$). Other breed differences in birth weight were not statistically significant, but several other comparisons, such as Columbia vs. Shropshire, Hamp. × SD. vs. Shropshire, and Hamp. × SD. vs. Dorset were large enough to be of practical importance. Miller (1935) reported that lambs sired by Hampshire rams were heavier at birth than those sired by Suffolks or Southdowns. Kincaid (1943) found a difference of 1.05 lb. between Hampshire and Southdown sired lambs. Ne-

TABLE 2. LEAST SQUARES ESTIMATES AND STANDARD ERRORS OF THE INFLUENCE OF BREED OF SIRE, SEX AND TYPE OF BIRTH

Item	Birth wt., lb.	Head circum., in.	Chest circum., in.	Wither ht., in.
Hampshire	0.72±.11	0.21±.05	0.27±.08	0.55±.07
Southdown	0.00	0.00	0.00	0.00
Shropshire	0.17±.24	0.18±.10	0.07±.17	0.49±.15
Dorset	−.01±.21	−.04±.09	−.08±.15	0.45±.13
Columbia	0.75±.27	0.24±.12	0.47±.19	1.14±.17
Corriedale	0.27±.33	0.26±.14	0.02±.23	0.68±.20
Hamp. × S.D.	0.69±.25	0.33±.11	0.48±.18	0.45±.16
Ewes	−.36±.09	−.08±.04	−.19±.07	−.25±.06
Rams	0.00	0.00	0.00	0.00
Singles	0.00	0.00	0.00	0.00
Twins	−1.88±.10	−.51±.04	−1.07±.07	−.78±.01
Years and age of dam not shown				
Est. of mean	9.00	10.27	14.13	13.19

TABLE 3. ADJUSTED MEANS BY BREED OF SIRE[a]

Breed	No. lambs	Birth wt., lb.	Head circum., in.	Chest circum., in.	Wither ht., in.
Hampshire	427	9.73	10.62	14.71	13.98
Southdown	444	9.01	10.41	14.44	13.43
Shropshire	58	9.18	10.59	14.51	13.92
Dorset	75	9.00	10.37	14.36	13.88
Columbia	44	9.76	10.65	14.91	14.57
Corriedale	26	9.28	10.67	14.46	14.11
Hamp. × S.D.	51	9.70	10.74	14.92	13.88

[a] Adjusted for type of birth, sex, age of dam, and year.

ville *et al.* (1958) reported no significant birth weight difference between lambs sired by Hampshire and Shropshire rams.

Lambs by the Hampshire × Southdown crossbred rams were slightly larger in head circumference than any of the other breeds studied, and significantly larger (about 1/3 inch) than those by Southdowns and Dorsets ($P<.01$). Those by Hampshire ($P<.01$) and Columbia ($P<.05$) rams were significantly larger than lambs sired by Southdowns. Miller (1935) reported that lambs sired by Hampshire rams were larger in head circumference than those by Suffolks, Shropshire, Southdown, or Rambouillet rams. The differences he reported were small but statistically significant.

Adjusted chest circumference of the lambs ranged from 14.92 inches for those by Hampshire × Southdown rams to 14.36 inches for Dorset sired lambs. Lambs by Hampshire × Southdown ($P<.01$), Hampshire ($P<.05$), and Columbia ($P<.01$) rams were significantly larger than those by Dorsets. A number of other comparisons, namely, Hampshire vs. Southdown, Columbia vs. Southdown, Hamp. × SD. vs. Southdown, Hamp. × SD. vs. Shropshire, Hamp. × SD. vs. Corriedale, and Corriedale vs. Columbia, were considered to be real breed differences, even though not significant at the 5% probability level. Miller (1935) reported that lambs by Hampshire and Suffolk rams were about equal in chest circumference and both were significantly

TABLE 4. BREED OF SIRE COMPARISONS OF BIRTH WEIGHT AND LINEAR MEASUREMENTS (BREED LISTED ACROSS THE TOP MINUS BREED LISTED AT THE LEFT)

Breed of sire	Character	Hampshire	Shropshire	Dorset	Columbia	Corriedale	Hamp. × S.D.
Southdown	Birth wt.	0.72±.11**	0.17±.24	−.01±.21	0.75±.27**	0.27±.33	0.69±.25**
	Head cir.	0.21±.05**	0.18±.10	−.04±.09	0.24±.12*	0.26±.14	0.33±.11**
	Chest cir.	0.27±.08**	0.07±.17	−.08±.15	0.47±.19	0.02±.23	0.48±.18
	Wither ht.	0.55±.07**	0.49±.15**	0.45±.13**	1.14±.17**	0.68±.20**	0.45±.16**
Hampshire	Birth wt.	.....	−.55±.24*	−.73±.22**	0.03±.26	−.45±.33	−.03±.26
	Head cir.	.....	−.03±.11	−.25±.10	0.03±.12	0.05±.14	0.12±.11
	Chest cir.	.....	−.20±.17	−.34±.15*	0.20±.19	−.25±.23	0.21±.18
	Wither ht.	.....	−.06±.15	−.10±.13	0.59±.16**	0.13±.20	−.10±.16
Shropshire	Birth wt.	.....	.....	−.18±.27	0.58±.35	0.10±.39	0.52±.29
	Head cir.	.....	.....	−.22±.12	0.06±.15	0.08±.17	0.15±.13
	Chest cir.	.....	.....	−.15±.19	0.20±.25	−.05±.28	0.41±.21
	Wither ht.	.....	.....	−.04±.17	0.65±.22**	0.19±.24	−.04±.18
Dorset	Birth wt.	.....	.....	.....	0.76±.33*	0.10±.37	0.52±.28
	Head cir.	.....	.....	.....	0.28±.15	0.30±.16	0.37±.12**
	Chest cir.	.....	.....	.....	0.55±.24**	0.10±.26	0.56±.20**
	Wither ht.	.....	.....	.....	0.69±.21**	0.23±.23	0.00±.18
Columbia	Birth wt.	.....	.....	.....	.....	−.48±.39	−.06±.36
	Head cir.	.....	.....	.....	.....	0.02±.17	0.09±.16
	Chest cir.	.....	.....	.....	.....	−.45±.28	0.01±.25
	Wither ht.	.....	.....	.....	.....	−.46±.24	−.69±.22**
Corriedale	Birth wt.	.....	.....	.....	.....	.....	0.42±.40
	Head cir.	.....	.....	.....	.....	.....	0.07±.18
	Chest cir.	.....	.....	.....	.....	.....	0.46±.29
	Wither ht.	.....	.....	.....	.....	.....	−.23±.25

* $P<.05$.

** $P<.01$; $s_{ij}=\sqrt{(c_{ii}+c_{jj}-2c_{ij})s^2}$.

TABLE 5. LEAST SQUARES ESTIMATES OF BREED OF SIRE DIFFERENCES INDEPENDENT OF TYPE OF BIRTH, SEX, AGE OF DAM, AND YEAR AND FOR A CONSTANT BIRTH WEIGHT

Breed of sire[a]	Head circum., in.	Chest circum., in.	Wither ht., in.
Hampshire	.01±.04	−.17±.04	.23±.05
Southdown	.00	.00	.00
Shropshire	.14±.08	−.03±.09	.41±.10
Dorset	−.04±.07	−.07±.08	.45±.09
Columbia	.03±.09	.02±.10	.81±.12
Corriedale	.19±.11	−.14±.13	.56±.14
Hamp. × S.D.	.13±.09	.07±.10	.14±.11
Partial regression on birth weight	.28±.01	.60±.01	.44±.01

[a] Other constants not shown.

larger than those sired by Southdown rams. Neville *et al.* (1958) found Shropshire sired lambs significantly smaller in shoulder circumference than those by Hampshires.

Lambs sired by Columbia rams averaged 14.6 inches in wither height and were significantly ($P<.01$) taller than those by any other breed of ram studied except Corriedale. Southdown lambs averaged 13.4 inches and were significantly lower than any of the other breeds ($P<.01$). Differences among the other breeds appeared to be negligible, except for Corriedale vs. Columbia, which was considered real.

One of the objectives of the study was to determine if a proportional difference in body dimensions, and particularly in head and chest circumference, existed between lambs sired by certain breeds of rams. Inspection of the adjusted means (table 3) indicated that birth weight, head and chest circumference, and perhaps wither height, were all expressions of general size, and that the linear measurements might be highly correlated with birth weight. It therefore seemed desirable to compare the lambs by breed of sire, with birth weight held constant. This was done by including birth weight in the linear least squares model as a continuous independent variable. Least squares estimates of the breeds of sire differences, independent of type of birth, sex, age of dam, year of birth, and for a constant birth weight are shown in table 5. Adjusted means by breed of sire for head and chest circumference and wither height were computed and are shown in table 6.

TABLE 6. ADJUSTED[a] MEANS BY BREED OF SIRE WITH BIRTH WEIGHT HELD CONSTANT

Breed of sire	Head circum., in.	Chest circum., in.	Wither ht., in.
Hampshire	10.35	13.87	13.36
Southdown	10.35	13.94	13.05
Shropshire	10.18	13.63	13.25
Dorset	10.06	13.68	13.36
Columbia	10.10	13.72	13.65
Corriedale	10.20	13.44	13.35
Hamp. × S.D.	10.21	13.79	12.99

[a] Adjusted for type of birth, sex, age of dam, year and partial regression on birth weight.

The analyses of variance (tests of differences shown in tables 3 and 6) due to differences between the adjusted breed of sire means, on the one hand with birth weight as a dependent variable, and on the other hand with birth weight as an independent variable, are shown in table 7. Breed differences for the three linear measurements were highly significant when birth weight was unaccounted for (Part A). A highly significant amount of variability in the three linear measurements was found to be due to regression on birth weight (Part B). When birth weight was held

TABLE 7. ANALYSES OF VARIANCE

Source of variation	Df	Mean squares			
		Birth wt.	Head circum.	Chest circum.	Wither ht.
A.[a]					
Regression	25	55.41**	7.85**	26.11**	24.41**
Breed of sire	6	20.90**	2.28**	4.51**	14.87**
Residual	1099	2.30	0.44	1.15	0.87
B.[b]					
Regression	26	.....	14.99**	59.70**	40.79**
Partial regr. on birth weight	1	.....	193.60**	899.31**	500.46**
Breed of sire	6	.....	0.36	1.10**	6.03**
Residual	1098	.....	0.26	0.34	0.42

[a] A, includes fitting constants for sex, age of dam, type of birth, breed of sire and year.
[b] B, includes fitting constants for sex, age of dam, type of birth, breed of sire, year and partial regression on birth weight.
** $P<.01$.

constant, breed differences in head circumference were no longer significant; breed differences in chest circumference and wither height were greatly reduced but remained highly significant.

Summary and Conclusions

Records of 1125 lambs were used to study the effects of seven different kinds of rams on weight, head circumference, chest circumference, and wither height of mutton type lambs at birth.

The least squares model included breed of sire as the effect of primary interest, and sex, type of birth, years and age of dam as environmental effects. A second model included all the effects mentioned, plus birth weight as a continuous independent variable.

A number of breed of sire differences were found to exist in the four traits studied. However, no proportional difference in head circumference due to breed of sire was demonstrated. Most of the breed differences in chest circumference were expressions of general size, although some proportional differences may exist. Much of the differences in wither height appeared to be characteristic of the breeds.

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COMPARATIVE FEEDING VALUE OF LACTIC ACID AND GRAIN FOR DAIRY CATTLE [1]

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LACTIC acid, as a constituent of silage, is a common feed for dairy cattle. Bentley *et al.* (1956) reported efficient gains in lambs fed lactic acid. Johnson *et al.* (1959) and Klosterman *et al.* (1959) observed increased gain and feed efficiency in sheep and steers fed rations fortified with lactic acid. However, their results were variable.

The present paper reports investigations designed to test the feed value of lactic acid for dairy cattle and its effect upon appetite.

Experimental Procedure

Metabolism Trials. Four dry Holstein cows were used for digestion and nitrogen balance trials of 4 by 4 Latin square design. Collections were made during the last 7 days of each 17-day period. The rations consisted of: (1) 25 lb. hay, (2) 17.6 lb. hay plus 5 lb. grain mixture, (3) 17.6 lb. hay plus 1.9 lb. lactic acid plus 2.5 lb. grain mixture, (4) 17.6 lb. hay plus 3.75 lb. lactic acid. These rations were estimated to contain the same amount of digestible energy. The hay was field cured, second cut alfalfa with a high weed content. It was field cured without rain and contained 14.1% crude protein. The grain mixture contained 83% ground corn, 15% soybean oil meal, 1% trace mineralized salt and 1% dicalcium phosphate. The lactic acid was 85% U.S.P. grade; it was assumed to contain 85% digestible nitrogen-free extract for purposes of computing the results. It was diluted with an equal weight of water and poured on the hay at the time of feeding. Blood glucose was determined on jugular blood drawn on the first and last day of each collection period 3 hr. after the morning feeding which consisted of one-half of the ration. Blood was preserved with sodium flouride, thymol, and potassium oxalate and was stored no longer than 48 hr. before analysis by the method of Somogyi (1945). Feed and fecal samples were analyzed by A.O.A.C. (1950) methods.

Growth Trial. Two groups of 10 heifers (8 Holsteins, 2 Brown Swiss) were used to study the effect of lactic acid on appetite, growth, and feed effiicency. Average initial weight of the heifers was 863 lb. for Group I and 866 lb. for Group II, individual weights ranging from 610 to 1089 lb. The heifers of Group I were each fed 2 lb. of corn daily during the first 14-day period, the corn being reduced to 1 lb., 0.5 lb., 0.25 lb. during the subsequent 14-day periods. During these same periods, heifers of Group II were each offered 2 lb. of 85% lactic acid which was subsequently reduced to 1 lb., 0.5 lb. and 0.25 lb. The lactic acid was diluted with an equal weight of water and mixed with the silage. Corn silage and hay were fed to appetite in both groups since feed refusals ranged from 5% to 50% of that offered. During a final 9-day period, the heifers of Group II were each fed 1 lb. of a mixture of sodium and calcium lactates while heifers of Group I were each fed 1 lb. corn. The lactate salts contained about 50% organic matter and only this organic matter was used in computing feed intake and efficiency. Hay and corn silage were the same as in the earlier periods. Gains were computed from weights taken on three consecutive days immediately before the trial began and at the end of each period.

Fermentation Trials. A series of five trials was conducted to determine the rate of destruction of lactic acid incubated with rumen microorganisms. Four Holstein cows equipped with rumen fistulas were used as sources of innocula. These cows were fed alfalfa hay and a corn-soy grain mixture. The rations ranged from 50% to 80% grain, and for one cow 2 lb. of lactic acid diluted with water was poured over the hay. The incubation mixture contained 150 ml. of rumen liquid strained through cheese cloth and 50 ml. of pH 7,

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M/10 phosphate buffer containing sufficient lactic acid to give a final concentration of 1000 to 1500 mcg./ml. In three trials, innocula were also prepared by a light centrifugation (about 150 × G for 5 min.) which removed the feed particles and protozoa. In one additional trial with centrifuged innocula, the lactic acid concentration was only 114 mcg./ml. Thus, in a total of six trials, dissimilation of lactic acid was replicated five times with strained rumen liquid and four times with centrifuged rumen liquid. Incubations were carried out in stoppered Erlenmeyer flasks at 39° C. for 4 hr. Lactic acid was determined by the method of Barker and Summerson (1941).

Results and Discussion

The apparent digestibilties of the four rations did not appreciably vary as shown in table 1. The greater digestibility of the ether extract in the corn containing rations can probably be attributed to the high digestibility of corn oil. The high digestibility of the nitrogen-free extract (NFE) of ration 4 (3.75 lb. lactic acid) supports the assumption that lactic acid is highly digestible. Although lactic acid is ether soluble, it probably occurs as a salt in feces and hence would appear in the ash and NFE fraction of the proximate analysis.

Blood sugar was not altered by feeding lactic acid but was increased by feeding grain (table 2). Hueter *et al.* (1956) observed an increase in blood glucose when two cows were given 3 lb. of lactic acid via rumen fistulas. The hyperglycemic effect persisted for 8 hr. When these investigators administered the lactic acid as its less soluble calcium salt, there was little change in the blood glucose level although

TABLE 1. APPARENT DIGESTIBILITY OF NUTRIENTS IN RATIONS

	1	2	3	4
Ration designation	Hay	Hay, grain	Hay, grain, lactic acid	Hay, lactic acid
Digestibility, %				
Dry matter	64.0	68.3	66.7	69.0
Organic matter	64.8	69.5	67.8	70.3
Crude protein	73.5	74.2	70.8	72.2
Ether extract[a]	20.9	48.8	27.4	24.8
Crude fiber	61.0	59.3	58.3	59.0
NFE[b]	67.2	76.1	75.2	78.8

[a] Significantly greater in ration 2 than in the other three rations at 5% level.
[b] Less in ration 1 than in 2, 3, or 4 and less in 3 than in 4 at 5% level of significance.

TABLE 2. BLOOD GLUCOSE OF COWS
(Mg. per 100 ml.)

Ration	Hay	Hay, grain	Hay, grain, lactic acid	Hay, lactic acid
1st day of period	52	47	49	48
Last day of period	48	51*	51*	48

* Significantly greater than the other two rations on last day of period at 5% level.

blood lactic acid increased. Hueter *et al.* (1956) and Waldo and Schultz (1956) reported that lactic acid is fermented chiefly to propionic acid by rumen bacteria, whereas Jayasuriya and Hungate (1959) found acetate as the predominant end product of this fermentation. The propionate production would be energetically more efficient and also glucogenic compared to acetate production.

The amount of lactic acid destroyed in the five fermentation trials with strained rumen liquid averaged 880 mcg. per ml. incubation mixture per 4 hr. with a standard deviation of 190. In three of the trials where centrifuged rumen liquid was used, destruction averaged 190 mcg./ml./4 hr. with a standard deviation of 70. The protozoa and/or easily sedimented bacteria appear to be responsible for a large share of the lactic acid metabolism in the rumen. In the fourth trial with centrifuged liquid where the initial concentration was 114 mcg./ml., 94 mcg. lactic acid/ml. were destroyed. Since approximately 50% of the lactate was destroyed at this lower substrate concentration whereas less than 20% was destroyed at the higher substrate concentration, it appears that the lactate fermenting system of the centrifuged liquid can be easily saturated. Glucose seems to be fermented several times faster than lactic acid by rumen contents (Waldo and Schultz, 1956).

Lactic acid depressed feed intake ($P=0.01$) and gain ($P=0.05$) in the heifer trial (table 3, periods I-IV). Lactic acid depressed feed efficiency in period IV ($P=0.01$) and apparently increased it in period I ($P=0.20$). Figure 1 indicates that feed efficiency increased in direct proportion to the lactic acid intake. Figures 1 and 2 suggest that under some conditions of feed and lactic acid intake, the increase in efficiency would more than compensate for the depressed feed intake. This condition was realized during period I. The lactate salts appeared to increase feed efficiency in the same manner as the free acid, but salts

TABLE 3. EFFECT OF LACTIC ACID ON WEIGHT GAINS, APPETITE AND FEED EFFICIENCY OF HEIFERS

Period	Ration	Supplemental lactic acid, lb.	Av. daily gain, lb.	Dry matter intake, lb.		Feed efficiency (gain/intake)
				Silage	Total	
I	Control (2 lb. corn)	0	1.8(0.3)[a]	7.8	20.6(0.4)	0.09(0.02)
	Lactic acid	0.8	1.9(0.3)	2.6	15.2(0.5)	0.12(0.02)
II	Control (1 lb. corn)	0	2.1(0.3)	7.8	21.4(0.5)	0.10(0.01)
	Lactic acid	0.6	1.8(0.2)	3.3	17.1(0.4)	0.10(0.01)
III	Control (0.5 lb. corn)	0	1.8(0.2)	7.4	20.6(0.4)	0.09(0.01)
	Lactic acid	0.4	1.2(0.3)	4.5	18.0(0.3)	0.07(0.02)
IV	Control (0.25 lb. corn)	0	1.6(0.2)	7.8	21.2(0.4)	0.08(0.01)
	Lactic acid	0.2	0.7(0.2)	5.3	18.3(0.3)	0.04(0.01)
V	Control (1 lb. corn)	0	1.0(0.2)	7.8	21.7(0.5)	0.04(0.01)
	Sodium and calcium lactate[b]	0.5	1.8(0.4)	5.7	19.2(0.5)	0.09(0.02)

[a] Numbers in parentheses are the standard error of the means.
[b] Computed as lactic acid.

were not as severe an appetite depressant as the free acid. The slow ruminal breakdown of lactic acid suggests that a systemic effect is responsible for the increased feed efficiency but does not preclude a ruminal effect.

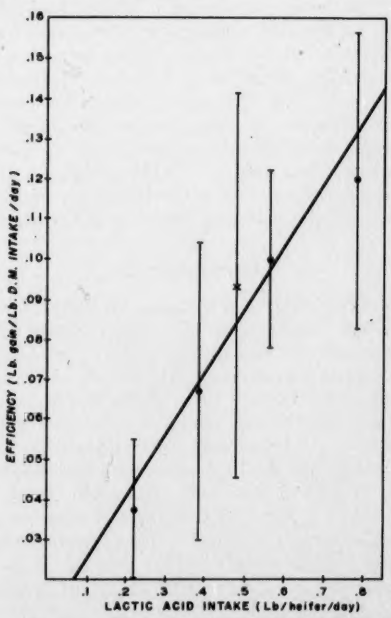

Figure 1. The relationship between feed efficiency and lactic acid intake. (The vertical lines indicate the 95% confidence range.)

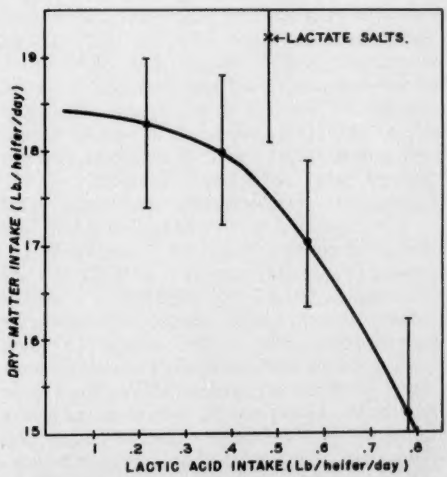

Figure 2. The relationship between lactic acid intake and appetite. (The vertical lines indicate the 95% confidence range.)

Summary

The nutritive value of lactic acid was studied in a digestion trial, a growth and appetite trial with heifers, and in fermentation trials with rumen contents *in vitro*. Lactic acid was fermented slowly by rumen contents but appeared to be entirely digested *in vivo*. Lactic acid decreased appetite in proportion to its concentration which probably explained its

slight depressing effect on milk production. Lactic acid markedly increased feed efficiency in proportion to its intake.

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EFFECT OF COPPER SULFATE AND OTHER CHEMOTHERAPEUTICS IN GROWING SWINE RATIONS [1]

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EVVARD *et al.* (1928) reported a beneficial response from copper sulfate when used as a feed additive for swine rations. Carpenter (1947) observed that growing pigs fed 45 ppm of supplemental copper gained faster than pigs fed the control ration. More recently Barber *et al.* (1957) presented an extensive study and cited other ones on the effect of high copper supplementation of diets for growing-finishing swine. Their work indicated no permanent deleterious effect from feeding 5 or 10 times the effective level (250 ppm Cu) for short periods of time (36 and 19 days, respectively). It indicated also that if the diet contains excessive copper in the sulfate form the pigs will compensate for this by reducing their feed intake. Copper at a level of 250 ppm improved performance throughout the experimental period which began when the pigs on test weighed about 40 lb. and ended when they weighed about 200 lb.

The experiments reported herein were conducted to study further the value of copper sulfate ($CuSO_4 \cdot 5H_2O$) at high levels and to compare its effectiveness as a growth stimulant with that of other chemotherapeutics.

Experimental

This study consisted of four trials involving 528 crossbred pigs. The experiments were of a randomized block design and the pigs were allotted to treatments from outcome groups of similar initial weight with the restriction that littermates be distributed across treatments. The experiments were conducted in dirt lots, and pigs had free access to feed and water at all times. The basal ration used in each of the experiments is presented in table 1.

Experiment 1. Four levels of copper sulfate (0, 0.05, 0.1 and 0.15% $CuSO_4 \cdot 5H_2O$) were added to the basal ration and the ration treatments were replicated three times with eight pigs per pen. Daily gain and feed required per pound of gain were recorded for the 42-day experimental period.

Experiment 2. A 2 x 2 factorial arrangement of 0 and 0.1% copper sulfate and 0 and 0.0025% 3-nitro-4-hydroxyphenylarsonic acid (3N4HPAA) treatments with three replications of eight pigs per pen was tested. Daily gain and feed per pound of gain were summarized on the 42nd and 107th day of the experiment. Individual fecal samples were collected from 18 pigs each of the basal, 3N4HPAA, and copper sulfate treatments after the pigs had been on experiment for 90 days. The samples were assayed for the following groups of organisms according to the method described by Difco Laboratories, Inc. (1953); coliform, lactobacilli, total aerobes, total anaerobes, staphylococci, streptococci and molds and yeasts.

Experiment 3. Six ration treatments including (1) basal, (2) copper sulfate, (3) copper sulfate plus nystatin, (4) copper sulfate plus rimocidin, (5) copper sulfate plus phytoactin and (6) copper sulfate plus phytostreptin were replicated three times with eight pigs per pen. Each of the antifungal antibiotics was added at the level of 30 mg. per pound of feed and copper sulfate was added at 0.1% in all cases. Daily gain and feed required per pound of gain were recorded for a 56-day experimental period. Fecal samples were assayed from 54 pigs (9 pigs per treatment) during the third week of the experiment and again from the same 54 pigs during the 7th week of the experiment.

Experiment 4. Six ration treatments including (1) basal, (2) oleandomycin (5 mg./lb.), (3) oxytetracycline (25 mg./lb.), (4) copper sulfate (0.1%), (5) treatments 2 plus 4, and (6) treatments 3 plus 4 were compared in an incomplete factorial arrangement of treatments with four replications of eight pigs per pen. Daily gain and feed required per pound of gain were summarized for the 42-day experimental period. Individual fecal samples

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[3] Department of Animal Husbandry.

TABLE 1. COMPOSITION OF BASAL RATION

Ingredient[a]	Pounds
Ground yellow corn	78.55
Solvent soybean oil meal (50% protein)	17.00
Vitamin premix[b]	2.00
Calcium carbonate	0.55
Dicalcium phosphate	1.30
Iodized salt	0.50
Trace mineral premix[c]	0.10

[a] In Experiment 1, condensed fish solubles was added at the level of 2.5% of the ration and corn and soybean oil meal were replaced to maintain a 16% protein ration.
[b] Supplied supplemental vitamins so that total ration was calculated to contain 2000 I.U. vitamin A, 400 I.U. vitamin D_3, 2.5 mg. riboflavin, 6.0 mg. pantothenic acid, 20 mg. niacin, 380 mg. choline and 10 mcg. vitamin B_{12} per pound.
[c] Supplied the following in ppm of complete ration: iron, 70.4; copper, 4.8; cobalt, 1.6; zinc, 81.6; manganese, 56.8.

were collected from 14 pigs on each treatment during the 5th week of the experiment and analysed by the method previously mentioned. Eight pigs on the basal ration and 14 pigs on the copper sulfate treatment were continued on the same rations until they averaged 125 lb. of body weight. At this time the protein content was lowered from 16 to 12% and six of the pigs that had been fed the copper sulfate supplemented ration were placed on the basal ration. As the pigs reached market weight they were slaughtered and the liver and loin from each animal were assayed for copper by the method described by Andrus (1955). The tissue samples for the copper analysis were obtained from the homogenized whole livers and from one-pound samples of homogenized loin from which the separable fat had been removed.

Results and Discussion

Experiment 1. The results of experiment 1 are presented in table 2. The regression analysis revealed a significant linear regression of feed/gain on copper sulfate levels at P=0.10 or less. The maximum response for both gain and feed per pound of gain was obtained at the 0.1% level of copper sulfate.

TABLE 2. EFFECT OF COPPER SULFATE ON RATE OF GAIN AND POUNDS FEED REQUIRED PER POUND OF GAIN (EXPERIMENT 1)

Level of $CuSO_4 \cdot 5H_2O$ (%)	0	0.05	0.10	0.15
No. of pigs per pen	8	8	8	8
No. of pens per treatment	3	3	3	3
Initial wt., lb.	36.6	36.4	37.0	35.9
Av. daily gain, lb.	1.32	1.35	1.44	1.38
Feed/gain, lb.[a]	2.79	2.75	2.66	2.68

[a] Linear regression of feed/gain on Cu levels significant (P=0.10 or less).

Experiment 2. The results of this experiment are presented in table 3. Analysis of gain made the first 42 days revealed a significant (P=0.01 or less) improvement in gain and feed conversion due to copper sulfate. Neither the 3N4HPAA effect nor the interaction between copper sulfate and 3N4HPAA was significant. Three poor doing pigs, one from the basal treatment and two from the copper sulfate treatment, were removed from the experiment during this period. Because of the limited shade facilities two pigs were removed from each pen after 42 days and the remaining six pigs were continued to market weight. After

TABLE 3. EFFECT OF COPPER SULFATE AND 3-NITRO-4-HYDROXYPHENYLARSONIC ACID ON RATE OF GAIN, POUNDS FEED REQUIRED PER POUND OF GAIN AND FECAL FLORA COUNTS (EXPERIMENT 2)

Treatment	Basal	3N4HPAA[a]	$CuSO_4$[b]	3N4HPAA + $CuSO_4$
No. pigs per pen	8	8	8	8
No. pens per treatment	3	3	3	3
Initial wt., lb.	25.9	25.9	26.4	26.1
Av. daily gain, lb.				
42-day summary[c]	0.95	1.00	1.22	1.21
107-day summary[d]	1.43	1.44	1.59	1.55
Feed/gain, lb.				
42-day summary[c]	2.69	2.55	2.45	2.37
107-day summary	3.27	3.18	3.14	3.07
Fecal organisms—log no. per gram of feces				
Coliforms[d]	6.59	6.40	7.03	....
Lactobacilli[c]	8.87	8.97	8.27	....
Total aerobes[c]	8.76	8.87	8.08	....
Total anaerobes	9.26	9.23	8.91	....
Staphylococci	6.28	6.12	5.92	....
Streptococci	7.77	7.34	7.01	....
Molds and yeasts[d]	5.38	5.25	5.72	....

[a] 3-nitro-4-hydroxyphenylarsonic acid (0.0025%).
[b] $CuSO_4 \cdot 5H_2O$ (0.10%).
[c] $CuSO_4 \cdot 5H_2O$ vs. none significant (P=0.01 or less).
[d] $CuSO_4 \cdot 5H_2O$ vs. none significant (P=0.05 or less).

the pigs had been on experiment for 90 days fecal counts were obtained on seven classes of organisms. Each value is recorded as the log count per gram of fresh feces. The 3N4HPAA treatment did not significantly affect any class of organisms. However, copper sulfate significantly (P=0.05 or less) increased the coliforms and molds and yeasts, and decreased the streptococci. Copper sulfate also decreased lactobacilli and total aerobes significantly (P =0.01 or less). It is of interest to note that copper sulfate increased the molds and yeasts much the same as has been reported for broad spectrum antibiotics (Pappenfort and Schnall, 1951). These effects of copper sulfate on the flora populations may be responsible for its stimulating effects on gains and feed conversion. The 107-day gain and feed conversion data showed an improvement due to copper

TABLE 4. THE EFFECT OF COPPER SULPHATE AND ANTIFUNGAL ANTIBIOTICS ON RATE OF GAIN, POUNDS FEED REQUIRED PER POUND OF GAIN AND FECAL FLORA COUNTS (EXPERIMENT 3)

Treatment	Basal	$CuSO_4$[a]	$CuSO_4$ + Nyst.[b]	$CuSO_4$ + Phytoa.[b]	$CuSO_4$ + Phytos.[b]	$CuSO_4$ + Rimo.[b]
No. of pigs per pen	8	8	8	8	8	8
No. of pens per treatment	3	3	3	3	3	3
Av. initial wt., lb.	36.7	36.8	35.9	37.0	35.8	36.7
Av. daily gain, lb.	1.23[c]	1.37	1.37	1.36	1.43	1.39
Feed/gain, lb.	2.96	2.80	2.85	2.86	2.88	2.83
Fecal organisms—3rd week on experiment—log no. per gram of feces						
Coliforms	5.77	6.83	6.91	6.68	7.07	6.75
Lactobacilli	9.07[c]	8.03	8.35	7.97	8.15	8.34
Total aerobes	8.22[d]	7.72	7.89	7.68	7.82	7.95
Total anaerobes	9.24[d]	8.64	8.83	8.85	8.64	8.97
Staphylococci	5.70	5.72	5.99	5.72	5.82	5.68
Streptococci	6.86	6.54	6.67	6.50	6.88	6.96
Molds and yeasts	5.51	5.67	5.82	5.56	5.95	6.10
Fecal organisms—7th week on experiment—log no. per gram of feces						
Coliforms	6.76	7.10	7.32	7.09	7.45	6.97
Lactobacilli	8.89[c]	8.07	8.07	8.31	7.88	8.11
Total aerobes	8.35[c]	7.61	7.88	7.78	7.79	7.66
Total anaerobes	9.07[d]	8.54	8.23	8.37	8.37	8.41
Staphylococci	5.39	5.69	5.68	5.75	5.69	5.71
Streptococci	7.36	6.91	6.78	6.98	6.42	6.85
Molds and yeasts	5.70	5.97	6.24	6.05	6.04	5.90

[a] $CuSO_4 \cdot 5H_2O$ (0.10%).
[b] Nystatin, phytoactin, phytostreptin and rimocidin (30 mg./lb. of feed).
[c] Differs significantly from others (P=0.01 or less).
[d] Differs significantly from others (P=0.05 or less).

sulfate. The effect of copper sulfate on rate of gain was statistically significant (P=0.05 or less). No effect on gain but an improvement in feed conversion was observed from the 3N4HPAA supplementation; however, the improvement in feed conversion was not statistically significant.

Experiment 3. Several antifungal antibiotics were combined with copper sulfate to study the effects on gain and feed conversion, and the effects on the fecal microorganisms, particularly the molds and yeasts. The results of this experiment are presented in table 4. Copper sulfate improved rate of gain and feed conversion significantly (P=0.01 or less). An improvement in rate of gain was noted only for phytostreptin of the antifungal antibiotics and this improvement was not statistically significant. Copper sulfate decreased lactobacilli, total aerobes and total anaerobes significantly (P=0.05 or less). A higher mold and yeast count was noted for the copper sulfate treated pigs as compared with the controls; however, this difference was not statistically significant. None of the antifungal antibiotics decreased the molds and yeasts count. A total of six poor doing pigs (two from the basal treatment, two from the copper sulfate-phytostreptin treatment and two from the copper sulfate-rimocidin treatment) were removed from the experiment during this period.

Experiment 4. Copper sulfate and two antibiotics (oleandomycin and oxytetracycline) were added separately and in combination. The results of this experiment are presented in table 5. Copper sulfate supplementation as compared with no copper sulfate increased rate of gain (P=0.05 or less). Also the antibiotics (oleandomycin and oxytetracycline vs. no antibiotic) increased rate of gain (P=0.01 or less). The statistical analysis of the feed conversion data showed a significant difference in feed required per pound of gain between the oleandomycin-treated pigs and the oxytetracycline-treated pigs (P=0.05 or less). It should be pointed out that two replications were conducted during the months of December and January, and due to the severe weather conditions it was impossible to keep the water in the automatic fountains from freezing. The automatic fountains in two pens of pigs on copper sulfate treatment and one pen on oleandomycin treatment were frozen more often than the others. This may partially

TABLE 5. EFFECT OF COPPER SULFATE, OLEANDOMYCIN AND OXYTETRACYCLINE ON GAINS, POUNDS FEED REQUIRED PER POUND OF GAIN AND FECAL FLORA COUNTS (EXPERIMENT 4)

Treatment	Basal	Oleand.[a]	Oxytet.[b]	$CuSO_4$[c]	$CuSO_4$[c] + Oleand.[a]	$CuSO_4$[c] + Oxytet.[a]
No. of pigs per pen	8	8	8	8	8	8
No. of pens per treatment	4	4	4	4	4	4
Initial wt., lb.	39.4	39.6	40.1	39.5	40.2	39.8
Av. daily gain, lb.	1.23	1.29	1.32	1.25	1.42	1.41
Feed/gain, lb.	3.10	3.19	2.98	3.19	3.03	2.91
Fecal organisms—log no. per gram of feces						
Coliforms	5.95	6.79	6.96	7.21	7.64	7.92
Lactobacilli	9.17	8.80	9.16	8.72	8.10	8.65
Total aerobes	8.78	8.49	8.84	8.12	7.86	8.50
Total anaerobes	9.29	9.10	9.27	9.00	8.59	9.10
Staphylococci	5.36	5.78	5.64	5.57	5.72	5.87
Streptococci	7.46	6.87	7.02	7.17	5.98	6.37
Molds and yeasts	5.80	5.82	6.13	5.18	6.26	6.40

[a] Oleandomycin (5 mg./lb. of feed), significantly increased rate of gain, coliforms and molds and yeasts and decreased feed required per pound of gain, lactobacilli, aerobes and anaerobes (P=0.05 or less).
[b] Oxytetracycline (25 mg./lb. of feed), significantly increased rate of gain, coliforms and molds and yeasts and decreased feed required per pound of gain (P=0.05 or less).
[c] $CuSO_4 \cdot 5H_2O$ (0.1%), significantly increased rate of gain and coliforms and decreased lactobacilli, aerobes, anaerobes and streptococci (P=0.05 or less).

account for the poorer performance of pigs on these two treatments alone (copper and oleandomycin) as compared with the combination of the two.

Tissue copper was determined on 22 animals. The copper content (ppm) of the dry matter of the liver and loin from each animal is recorded in table 6. There were no statistically significant differences in copper content of loin between pigs on the basal, copper sulfate to 125 lb. of body weight, and copper sulfate to market weight. However, there was a significant difference (P=0.01) in liver copper between pigs on the basal (29 ppm) and those on copper sulfate (293 ppm) to market weight. These tissue copper values agree closely with those reported by Barber *et al.* (1957) and Lucas and Calder (1957). Although the copper content of the liver of the treated animals is high, it is of about the same magnitude as the normal copper content of the liver of sheep (Albiston *et al.*, 1940).

TABLE 6. EFFECT OF COPPER SULFATE ON COPPER CONTENT OF LOIN AND LIVER SAMPLES (EXPERIMENT 4)

Treatment	Basal	$CuSO_4 \cdot 5H_2O$ (0.1%) to 125 lb.	$CuSO_4 \cdot 5H_2O$ (0.1%) to 200 lb.
Number of pigs	8	6	8
Loin copper, ppm of dry matter	5.8	6.5	7.3
Liver copper, ppm of dry matter	29	67	293[a]

[a] Significantly greater than others (P=0.01 or less).

The fecal microorganisms were considerably influenced by the various treatments. Copper sulfate significantly (P=0.01 or less) increased the coliforms and decreased the lactobacilli, total aerobes, total anaerobes and streptococci. The antibiotics significantly (P=0.01 or less) increased the coliforms and molds and yeasts, and the oleandomycin significantly (P=0.05 or less) lowered the lactobacilli, total aerobes, total anaerobes and streptococci. With the limited data it is difficult to differentiate between the action of copper sulfate and either of the antibiotics. However, it is obvious that copper sulfate exerted an effect on the fecal microflora which might account for the improved performance of these animals.

Summary

Four trials involving 528 crossbred pigs were conducted to study the effectiveness of copper sulfate as a feed additive in growing swine rations. The first trial indicated that 0.1% copper sulfate ($CuSO_4 \cdot 5H_2O$) is the optimum level for rapid growth rate and efficient feed conversion. The combination of copper sulfate and 3N4HPAA in the second trial resulted in responses no greater than copper sulfate alone. In that trial copper sulfate increased the fecal mold and yeast

count much as would an antibiotic. In the third trial copper sulfate resulted in an improved growth rate. None of the antifungal antibiotics (nystatin, phytoactin, phytostreptin or rimocidin) gave further improvement when added in combination with copper sulfate, and none of them depressed the fecal mold and yeast count. The combination of copper sulfate and either oleandomycin or oxytetracycline in the fourth trial appeared to give an additive effect with respect to growth rate. The tissue studies for copper showed no statistically significant differences in the copper content of the loin between pigs fed a ration containing 0.1 percent copper sulfate and those fed the control ration. However, this level of copper increased liver copper 10 fold (29 to 293 ppm). Copper sulfate significantly increased lactobacilli, total aerobes, total anaerobes and streptococci organisms. The antibiotics significantly increased the fecal count of coliforms and molds and yeasts and decreased the streptococci organisms.

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SOURCES OF UNIDENTIFIED GROWTH FACTORS FOR BABY PIGS [1]

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SINCE the isolation and identification of vitamin B_{12} by Rickes *et al.* (1948), workers in the fields of poultry and swine nutrition have reported unidentified growth factor (UGF) activity in virtually a host of feedstuffs. Scott (1957) reported that evidence from various workers indicates the existence of approximately five distinct growth factors for the chick. It would appear that for the pig there are four such factors, namely: (1) the fish factor (Noland *et al.*, 1954; Miner *et al.*, 1955); (2) the distillers dried solubles factor (Catron *et al.*, 1954); (3) the whey factor (Vohs *et al.*, 1951); and (4) the forage juice factor (Gard *et al.*, 1955). These studies were initiated to study the following materials for growth promoting activity in the pig: (1) distillers dried solubles [6] (DDS), (2) fish solubles [7] (FS), (3) corn steepwater (CSW), and (4) corn fermentation solubles [8] (CFS).

Experimental

The majority of pigs used in these experiments were from Farmers Hybrid [9] boars mated to three-way crossbred sows (Poland China, Landrace, and Duroc). The male pigs were castrated before one week of age and all pigs treated for anemia by intraperitoneal injection at one day of age with 2 cc of an iron-dextran complex (50 mg. Fe/cc). All pigs were injected with 10 cc of chlorea-erysipelas anti-serum prior to allotment to their respective experiments. The buildings were steam cleaned and thoroughly disinfected prior to use in each experiment.

In experiment I the pigs were housed in individual pens on wire floors. In other experiments they were housed on concrete-floored pens with three to four pigs per pen. Within the buildings a minimum ambient temperature of 70° F was maintained.

All pigs were allowed free access to feed and water. The compositions of the basal diets are presented in table 1. Basal diet A was used as a common pre-experimental diet for experiments I and III. Basal diet B was used for the first 28 days in all experiments. Diet C was used after 28 days in each experiment except experiment I in which the experimental period itself was only 28 days in length. The experimental rations were made up by substituting the growth factor source into the basal diets. The rations made from each basal were equalized on the basis of calculated protein, calcium, phosphorus and known vitamins by varying the levels of corn, soybean oil meal, calcium carbonate, dicalcium phosphate and supplemental vitamins. Weight gains and feed consumption were recorded weekly. The pen of

TABLE 1. PERCENTAGE COMPOSITION OF BASAL DIETS

Ration	A	B	C
Ground yellow corn	19.45	37.1	78.5
Cane sugar	15.0	15.0	....
Solvent soybean oil meal (50% protein)	16.7	5.0	17.0
Dried skimmed milk	40.0	40.0	....
Dried beet pulp	2.0	...	...
Stabilized lard	5.0	1.0	...
Vitamin-antibiotic premix [a, b]	0.2	0.2	2.0
Calcium carbonate	0.2	0.1	0.5
Dicalcium phosphate	0.8	1.0	1.4
Iodized salt	0.5	0.5	0.5
Trace minerals [c]	0.15	0.1	0.1

[a] Added at levels to give the following totals per pound of diet: A & B—vitamin A, 3000 I.U.; vitamin D_3, 1000 I.U.; riboflavin, 5 mg.; pantothenic acid, 10 mg.; niacin, 30 mg.; choline chloride, 450 mg.; vitamin B_{12}, 20 mcg.; alpha-tocopherol acetate, 10 mg.; ascorbic acid, 100 mg.; menadione, 0.5 mg.; thiamine, 3 mg.; pyridoxine, 0.6 mg.; PABA, 2 mg.; inositol, 390 mg.; folic acid, 0.8 mg.; biotin, 3 mcg.; antibiotic, 50 mg. Diet C had the following levels: vitamin A, 2000 I.U.; vitamin D_3, 400 I.U.; riboflavin, 2.5 mg.; pantothenic acid, 6 mg.; niacin, 20 mg.; choline chloride, 400 mg.; vitamin B_{12}, 10 mcg.; antibiotic, 30 mg.

[b] The antibiotics used were as follows: Exp. 3, chlortetracycline; Exp. 1, 3:2 ratio of chlortetracycline and penicillin; Exp. 2, 4 and 5, 1:3 ratio of penicillin and streptomycin.

[c] One-tenth percent contributes the following in p.p.m.: Fe, 70.4; Cu, 4.8; Co, 1.6; Zn, 81.6; Mn, 56.8; K, 7.5.

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[6] Corn distillers dried solubles.

[7] Condensed red fish solubles.

[8] Condensed fermented corn extractives.

[9] Controlled crossbred boars, Farmers Hybrid Hogs, Hampton, Iowa.

TABLE 2. EFFECT OF LEVELS OF CONDENSED FISH SOLUBLES ON AVERAGE TOTAL GAINS AND POUNDS FEED REQUIRED PER POUND OF GAIN (EXPERIMENT 1)

Level of fish soluble, %	0.0	1.5	3.0	6.0
Total gain, lb.[a]	17.4	20.1	19.8	21.9
Feed/gain[a]	2.05	1.91	1.80	1.71

[a] Linear regression of gain and feed/gain on levels of fish solubles significant (P=0.05 or less). Coefficients of variability for gain=18.6% and for feed/gain=13.8%.

pigs was considered the experimental unit for analysis of the data, and reference to statistical significance pertains to the probability level of 5% unless otherwise indicated. Certain of the details of design and description of ration variables are presented with the results for clarification.

Experiment I. Thirty-two pigs averaging 7.7 lb. body weight and 14.2 days of age were randomly allotted from litter mate outcome groups to four levels (0, 1.5, 3 and 6%) of fish solubles. The first 5 days after weaning all pigs were fed the pre-experimental diet A (table 1) after which time the diet was fed supplemented with increasing amounts of fish solubles.

As the level of fish solubles in the ration was increased from 0 to 6%, both gains and feed/gain showed a significant linear improvement (table 2).

Experiment II. Eighty pigs averaging 11.0 lb. body weight and 17.4 days of age were randomly allotted from littermate outcome groups to five ration treatments. The ration variables were 5% corn fermentation solubles (CFS), 3% fish solubles (FS), 4% corn steepwater (CSW) and 5% corn fermentation solubles plus 1% fish solubles.

The performance of all pigs was quite satisfactory with average 6-week gains of 34.1 lb. on 2.0 lb. of feed per lb. of gain. Those pigs receiving 3% FS gained faster than those in any of the other treatment groups (table 3). Additions of 5% CFS, 1% FS + 5% CFS or 4% CSW failed to improve performance under the conditions of this experiment.

TABLE 3. EFFECT OF VARIOUS UNIDENTIFIED GROWTH FACTOR SOURCES ON 6-WEEK GAINS AND POUNDS FEED REQUIRED PER POUND OF GAIN (EXPERIMENT II)

Ration[a]	Basal	5% CFS	3% FS	1% FS+ 5% CFS	4% CSW
Total gain, lb.[b]	34.0	33.5	36.8	33.1	33.4
Feed/gain[b]	1.98	1.97	1.96	1.98	2.09

[a] CFS=corn fermentation solubles, FS=fish solubles, CSW=corn steepwater.
[b] Coefficient of variability for gain=8.8% and for feed/gain=13.8%.

Experiment III. One hundred forty-four crossbred pigs were weaned at an average weight of 6.8 lb. and age of 8.8 days, maintained on a common prestarter ration (basal diet A) for one week and then randomly allotted from littermate outcome groups to eight replications of a randomized block design. Prior to allotment the litters of equal age were grouped into light weight litters averaging 7.5 lb. and heavy weight litters averaging 8.5 lb. This method of grouping provided pigs of differing average initial weights but of equal average initial ages. In each of replications 1, 2, 5 and 6, two pens of pigs were fed the basal diet (zero percent DDS) and the results from the two pens averaged to give the treatment performance within a replication. The ration treatments consisted of four levels (0, 2.5, 5 and 10%) of distillers dried solubles (DDS). The gains and feed required per pound of gain are summarized in table 4.

TABLE 4. EFFECT OF DISTILLERS DRIED SOLUBLES ON 5-WEEK GAINS AND POUNDS FEED REQUIRED PER POUND OF GAIN (EXPERIMENT III)

Level of DDS, %	0	2.5	5.0	10.0
Initial weight	Total gain (lb.)			
7.5 lb.	20.8	21.7	23.2	21.9
8.5 lb.	24.0	25.3	25.6	26.6
Average[a]	22.4	23.5	24.4	24.2
	Feed/gain			
7.5 lb.	1.95	1.86	1.78	1.78
8.5 lb.	1.90	1.95	1.80	1.88
Average[a, b]	1.92	1.90	1.79	1.83

[a] Coefficient of variability for gain=8.8% and for feed/gain=4.4%.
[b] Statistically significant quadratic regression of feed/gain on levels of D.D.S. (P=0.05 or less).

Average total gain increased as the level of DDS increased up to 5% and then appeared to plateau. With the lighter weight pigs (replications 1–4, initially averaging 7.5 lb.), 5% appeared to be the optimum level; whereas, maximum gains were observed for the heavier pigs (initially averaging 8.5 lb.) at the 10% level of DDS. The feed required per pound of gain demonstrated a significant quadratic response with the most efficient feed conversion at the 5% level of DDS.

Experiment IV. One hundred twenty pigs averaging 9.2 lb. body weight and 15.4 days

TABLE 5. EFFECT OF DISTILLERS DRIED SOLUBLES AND TRACE MINERALS ON TOTAL 6-WEEK GAINS AND POUNDS FEED REQUIRED PER POUND OF GAIN (EXPERIMENT IV)

Rations	Basal		5% DDS		5% DDS (Ashed) [a]	
	−TM	+TM	−TM	+TM	−TM	+TM
Total gain, lb.[b]	20.4	20.8	21.6	21.5	19.8	22.3
Feed/gain[b, c]	1.82	1.75	1.63	1.67	1.80	1.62

[a] Distillers solubles=8.4% ash.
[b] Coefficient of variability for gain=7.7% and for feed/gain=6.7%.
[c] Distillers solubles vs. no distillers solubles statistically significant (P=0.05 or less).

of age were randomly allotted from littermate outcome groups to the six ration treatments consisting of a basal, basal plus 5% DDS, and basal plus the level of ash equivalent to 5% DDS, each fed with and without supplemental trace minerals. Total gain and feed required per pound of gain during the 6-week experimental period are summarized in table 5.

Added trace minerals had no consistent effect on gains, but appeared to improve feed conversion (1.75 vs. 1.68 lb. feed/lb. gain). Both distillers dried solubles and its ash slightly improved gains (20.6 vs. 21.3); however, neither this response nor the effect of trace minerals was statistically significant. A statistically significant improvement in feed required per pound of gain due to the addition of DDS or its ash equivalent was observed (1.78 vs. 1.68).

Experiment V. In an experiment of a split plot design, 96 pigs averaging 9.9 lb. body weight and 17.2 days of age were randomly allotted to treatments by weight within litter. The presence or absence of iodinated casein in the diet constituted the main plot treatments and the sources of UGF (5% distillers dried solubles, 4% corn steepwater, and 3% condensed fish solubles) constituted the subplot treatments. The inclusion of iodinated casein in the diet at levels of 120 mg./lb. the first four weeks and 150 mg./lb. the last two weeks of the experiment significantly depressed growth and increased feed required per pound of gain. Three percent fish solubles significantly improved total gain, but, did not improve feed conversion. This response was similar for both main plot treatments (+ or −iodinated casein). The addition of 5% DDS or 4% CSW did not improve gains or feed conversion either in the presence or absence of iodinated casein (table 6).

Discussion

Lewis *et al.* (1957) reported that the baby pig is unable adequately to utilize plant proteins until approximately 6 weeks of age, and that a diet containing 40% dried skimmed milk supported optimum growth. Therefore, in an effort to circumvent protein availability rather than UGF being the limiting factor in the growth of baby pigs, all diets fed to pigs under 6 weeks of age in these experiments contained 40% dried skim milk.

The results of these experiments indicate that distillers dried solubles (DDS) contains some factor(s) other than that contained in the basal ration which affects both gain and feed conversion in the baby pig, the effect being greater on the latter. Although the presence or absence of trace minerals in the diet did not significantly affect this response to DDS, there was an indication that the response was less in the absence of trace minerals with the group receiving the ashed DDS than with the other groups.

Although the differences obtained with the ash of DDS were quite small, they support the work of Kurnick *et al.* (1957) who noted that approximately half of the growth response to DDS could be obtained with the ash fraction.

TABLE 6. EFFECT OF DISTILLERS DRIED SOLUBLES, CORN STEEPWATER AND FISH SOLUBLES ON 6-WEEK GAINS AND FEED EFFICIENCY (EXPERIMENT V)

Rations	−Iodinated casein				+Iodinated casein			
	Basal	5% DDS	4% CSW	3% FS	Basal	5% DDS	4% CSW	3% FS
Gain, lb.[a]	34.9	32.9	30.0	36.2	29.8	26.1	28.8	31.7
Feed/gain[a]	1.68	1.72	1.80	1.71	2.04	2.07	2.15	2.09

[a] Coefficient of variability for gain=11.2% and for feed/gain=7.0%.

Growth response of a rather low magnitude coupled with a relatively high normal variation would make it desirable to introduce a factor which might exaggerate treatment effects. Zinn and Anderson (1955) demonstrated that iodinated casein (164 mg./lb. diet) imposed observable symptoms of thyrotoxicosis on the growing pig and that 3 or 5% liver residue in the diet counteracted these effects but 5% fish solubles (FS) was ineffective. During the first 4 weeks of experiment III, iodinated casein at 120 mg./lb. of diet produced no definite thyrotoxic symptoms. At the end of the fourth week the level was increased to 150 mg./lb. of diet and this level resulted in thyrotoxic symptoms such as increased respiration rate, depressed gain and poorer feed conversion. Under the conditions of this experiment DDS and CSW were without effect in counteracting the thyrotoxic effects of iodinated casein, but the addition of 3% FS improved rate of gain both in the presence and absence of the iodinated casein.

The consistent improvement in animal performance with additions of FS in these experiments indicates the presence of UGF activity separate from any that might be present in the basal diets used. It would have been of interest to study levels higher than those used here (6% of the diet) but "balling up" of the feed prevented this. Results with the CSW and CFS demonstrate the adequacy of the basal diet for any possible UGF activity present in these sources.

Summary

Five experiments involving 472 baby pigs were conducted to study the growth promoting activity of various crude sources of unidentified growth factors. The optimum level of distillers dried solubles (DDS) was approximately 5% of the total diet, and a portion of this growth response could be attributed to the ash of DDS. The inclusion of trace elements (iron, copper, cobalt, manganese and zinc) in the diet did not appreciably affect this response.

Impaired performance resulting from the inclusion of iodinated casein in the diet was not overcome by the inclusion of DDS or corn steepwater but was partially overcome by the addition of fish solubles.

Fish solubles at increasing levels (0 to 6%) produced a linear improvement in 4-week gain and feed conversion. Corn steepwater and corn fermentation solubles did not improve performance.

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EFFECT OF PROTEIN LEVEL, AMINO ACID SUPPLEMENTATION AND DURATION OF FEEDING OF A DRY EARLY-WEANING PIG RATION

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THE original successful use by the authors of a dry ration for feeding pigs weaned at two weeks of age was contingent upon the ration containing relatively large quantities of costly protein supplements of high biological value (Crampton and Ness, 1954). Since that time, attempts have been made to reduce the cost of this early-weaning ration through ingredient substitutions and/or by reducing protein level.

The lack of success in replacing all the skimmilk powder in this ration by a mixture of meat meal and whey powder has already been reported (Lloyd and Crampton, 1958a). The decreased rate of gain and feed efficiency of pigs fed the rations containing meat meal and whey powder was shown to be due, at least in part, to a lower digestibility of the crude protein and total carbohydrate in these feeds. These results tended to confirm the findings of Lewis *et al.* (1955) that, for maximum efficiency, casein and lactose are essential in the ration of pigs under 5–6 weeks of age.

However, the study of Lloyd and Crampton (1958a) indicated that early-weaning rations containing skimmilk powder were as satisfactory for pigs when fed at a protein level of 26% as at the original 30% level. Subsequent trials, in which the skimmilk powder and total protein contents were reduced further, showed that rations containing 22% protein were not inferior to those containing 30% (Lloyd and Crampton, 1958b).

The deleterious effect of amino acid imbalances on ration efficiency are now well known, and have been ably summarized by Elvehjem (1956) and Harper and Kumta (1959). Therefore, the work reported herein deals with attempts to improve the efficiency of early-weaning pig rations through an improvement of amino acid balance. In addition, the effect of changing from an early-weaning to a standard 18% protein starter ration at different ages prior to 8 weeks was examined as an alternative in reducing the feed cost of the early-weaning program.

Experimental Procedure

A comparison of the essential amino acid composition of the original 30% protein early-weaning pig ration of Crampton and Ness (1954) with that of the United States NAS-NRC (1953) [3] requirements for pigs weighing 25–70 lb., and with that of dried whole egg protein, showed that it was unbalanced with respect to the amino acids, methionine and isoleucine. When the proportions of the ingredients in the 30% protein ration were changed to produce a ration containing 20% protein, the same amino acid picture was found. A similar imbalance with respect to methionine and isoleucine was observed in a typical 18% protein starter ration. In all cases, the content of methionine and isoleucine was low relative to that of the other essential amino acids. This imbalance and the effect of subsequent amino acid supplementation is illustrated in table 1.

A total of 96 Yorkshire pigs, divided equally as to sex, were weaned and placed on test at 14 days of age in three replicates. The 32 pigs in each replicate were fed for a 6-week period. The pigs were allotted at random and penned individually in a suitably heated building.

Four ration treatments were used in each replicate. In ration treatment A, both a 30% and a 20% protein early-weaning ration were fed for the full 6-week period. In ration treatments B, C and D, both of these early-weaning rations were fed for the first 4, 3 and 2 weeks, respectively, followed by a change to an 18% protein starter ration for the remainder of the feeding period.

The composition of the rations fed is given in table 2. The addition of the indicated

[1] The authors are indebted to the Agricultural Research Council, Quebec Department of Agriculture for financial support in this study; and, to Merck and Company for the supply of amino acids and for part financial assistance.

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[3] This research was carried out prior to the publication of the 1959 revision of the swine nutrient requirements. Upon examination of this later edition, the same general picture with regard to methionine and isoleucine was found to hold true.

TABLE 1. ILLUSTRATION OF THE DL-METHIONINE AND L-ISOLEUCINE IMBALANCE OF THREE SWINE RATIONS AND THE EFFECT OF SUPPLEMENTATION WITH THESE AMINO ACIDS

Item	Amino acid as percent of total essential amino acids	
	DL-Methionine	L-Isoleucine
NAS-NRC requirements (25–70 lb. pig)	11.6	13.6
Dried whole egg protein	7.3	13.7
30% protein early-weaning ration	4.8	12.4
20% protein early-weaning ration	4.4	11.9
18% protein starter ration	3.9	11.7
30% protein early-weaning ration, supplemented	7.5	13.7
20% protein early-weaning ration, supplemented	7.6	13.6
18% protein starter ration, supplemented	7.8	13.8

amounts of isoleucine and methionine to the 30% and 20% protein early-weaning rations and to the 18% protein starter ration increased the proportion of these essential amino acids to within the range found in the NAS-NRC requirements or in dried whole egg protein (see table 1). Because of the scarcity and high price of L-isoleucine, twice the amount required was added in the form of DL-isoleucine.

All pigs were allowed to eat *ad libitum;* water was available at all times. Each pig was weighed at weekly intervals and the weekly feed consumption per pig was also recorded.

Results and Discussion

The relevant data on average daily gain, average daily feed consumption and feed efficiency are given in table 3.

The figures in Block 1, showing a significant decrease in the average daily gain and average daily feed consumption of pigs fed the 20% early-weaning rations, indicate that rations carrying 20% total crude protein are inadequate for pigs weaned at two weeks of age.

The data in Blocks 2 and 3 show that the correction of the methionine and isoleucine imbalances in any of the rations fed did not affect gain or increase the biological efficiency of such rations. Thus it must be concluded that the apparent amino acid imbalances were not of sufficient magnitude to have had any deleterious effect on the biological value of the rations.

The success of the attempts to reduce the feed cost of the early-weaning program by changing from an early-weaning to a standard 18% starter ration prior to 8 weeks of age is indicated by the results shown in Blocks 4 and 5. If differences in the protein content of the early-weaning rations are disregarded, the data in Block 4 show a steady decrease in average daily gain and feed intake as pigs are changed

TABLE 2. PHYSICAL COMPOSITION OF RATIONS

Ration ingredient	Ration number					
	30% Protein		20% Protein		18% Protein	
	1	2	3	4	5	6
	(%)	(%)	(%)	(%)	(%)	(%)
Wheat	10.0	10.0	22.0	22.0	....	....
Oat groats	15.0	15.0	38.0	38.0	....	....
Barley	....	....	....	....	42.0	41.2
Shorts	....	....	....	....	15.0	15.0
Middlings	....	....	....	....	25.0	25.0
Soybean oilmeal	10.0	10.0	6.0	6.0	6.0	6.0
Fishmeal	10.0	10.0	6.0	6.0	2.0	2.0
Meatmeal	....	....	....	....	5.0	5.0
Dried brewers' yeast	5.0	5.0	5.0	5.0	....	....
Dried skimmilk powder	37.0	37.0	10.0	10.0	2.0	2.0
Cane molasses	10.0	9.0	10.0	9.2	....	....
Vitamin-mineral-antibiotic supplement [a]	3.0	3.0	3.0	3.0	3.0	3.0
DL-methionine	....	0.43	....	0.34	....	0.35
DL-isoleucine [b]	....	0.54	....	0.48	....	0.50

[a] Composition given by Lloyd and Crampton (1958a).
[b] Double the required amount of L-isoleucine supplied in the form of DL-isoleucine.

TABLE 3. THE RESPONSE OF PIGS TO A CHANGE FROM EARLY-WEANING RATIONS TO A STARTER RATION AT DIFFERENT AGES, TO RATION PROTEIN LEVEL, AND TO AMINO ACID SUPPLEMENTATION

Block no.	Treatment	Average daily gain	Average daily feed consumption	Feed efficiency (feed/gain)
		(lb.)	(lb.)	(lb.)
1	30% Early-weaning rations	0.71	1.53	2.2
	20% Early-weaning rations	0.61	1.40	2.3
	L.S.D. (P=0.05)	0.06	0.12	...
2	30% Early-weaning rations	0.72	1.58	2.2
	30% Early-weaning+methionine+isoleucine	0.70	1.48	2.1
	20% Early-weaning rations	0.62	1.38	2.2
	20% Early weaning+methionine+isoleucine	0.61	1.41	2.3
	Maximum L.S.R.* (P=0.05)	0.10	0.20	...
3	No supplemental methionine+isoleucine	0.67	1.48	2.2
	Supplemental methionine+isoleucine	0.66	1.45	2.2
	L.S.D. (P=0.05)	0.06	0.12	...
4	Early-weaning (6 weeks)	0.74	1.63	2.2
	Early-weaning (4 weeks)+starter (2 weeks)	0.71	1.50	2.1
	Early-weaning (3 weeks)+starter (3 weeks)	0.66	1.44	2.2
	Early-weaning (2 weeks)+starter (4 weeks)	0.53	1.28	2.4
	Maximum L.S.R.* (P=0.05)	0.10	0.20	...
5	30% Early-weaning (6 weeks)	0.81	1.75	2.2
	30% Early-weaning (4 weeks)+18% starter (2 weeks)	0.80	1.55	1.9
	30% Early-weaning (3 weeks)+18% starter (3 weeks)	0.70	1.49	2.1
	30% Early-weaning (2 weeks)+18% starter (4 weeks)	0.53	1.34	2.5
	20% Early-weaning (6 weeks)	0.67	1.52	2.3
	20% Early-weaning (4 weeks)+18% starter (2 weeks)	0.63	1.45	2.3
	20% Early-weaning (3 weeks)+18% starter (3 weeks)	0.63	1.39	2.2
	20% Early-weaning (2 weeks)+18% starter (4 weeks)	0.53	1.23	2.3
	Maximum L.S.R.* (P=0.05)	0.15	0.30	...

* L.S.R.=Least Significant Range.

to a starter ration at 6, 5 and 4 weeks of age. The inadvisability of changing pigs to a starter ration at 4 weeks of age is indicated by the significantly decreased rate of gain and feed consumption and by the decrease in feed efficiency.

In Block 5, the inferiority of the 20% protein early-weaning rations is reaffirmed. However, it is of greater significance that when the 30% protein early-weaning ration was fed to pigs for 4 weeks followed by an 18% protein starter ration for 2 weeks, the average daily gain was the same as when the early-weaning ration was fed for the full 6 weeks. This equal gain was accompanied by an increased feed efficiency. A progressive decrease in average daily gain and feed consumption was noted when the pigs were changed to the starter ration after 3 and 2 weeks on the early-weaning ration.

The origin of the increased feed efficiency when pigs are changed from an early-weaning ration to an 18% protein starter ration at 6 weeks of age is of considerable interest. The data presented in table 4 show the similarity in daily gain, feed intake and feed efficiency between pigs on either ration treatment during the first 4 weeks of the test period. This was to be expected since only the 30% protein early-weaning ration was being consumed. However, during the last 2 weeks of the test period, it should be noted that the pigs fed the starter ration grew at the same rate as those fed the early-weaning ration while consuming a significantly smaller amount of feed. Therefore, the originally observed increased feed efficiency (table 3) was primarily a reflection of the greater feed efficiency of the 18% starter ration over the 30% early-weaning ration during the last 2 weeks of the test period (table 4).

In this search for the minimum level of protein in early-weaning pig rations that is compatible with maximum gain and feed efficiency, the authors have been unable to confirm original findings that a 30% protein level is superior to a 26% level. In fact, subsequent work has indicated that protein levels of 26%

TABLE 4. THE RESPONSE OF PIGS TO TWO RATION TREATMENTS DURING DIFFERENT INTERVALS OF THE 6-WEEK FEEDING PERIOD

Ration treatment	Average daily gain (lb.)		Average daily feed consumption (lb.)		Feed efficiency (feed/gain)	
	First 4 weeks	Last 2 weeks	First 4 weeks	Last 2 weeks	First 4 weeks	Last 2 weeks
30% Early-weaning (6 weeks)	0.64	1.18	1.21	2.96	1.89	2.59
30% Early-weaning (4 weeks) + 18% starter (2 weeks)	0.63	1.17	1.09	2.41	1.73	2.06

and 22% are as satisfactory in the efficient promotion of growth as the 30% level. The present experiment indicated that a 20% level of protein is inadequate in early-weaning pig rations.

Therefore, on the basis of these findings and in order to conform with the recommendation of the Swine Standard Committee of the United States NRC Committee on Animal Nutrition, the authors suggest a level of 24% crude protein in early-weaning pig rations. The composition of such a ration is given in table 5. That this ration is entirely satisfactory for pigs of this age category has been illustrated by Lloyd and Crampton (1959).

During the course of research on the nutrition of the early-weaned pig over the past 10 years, the authors have noted that at least as much, and often more feed is consumed by early-weaned pigs between 6 and 8 weeks of age than between 2 and 6 weeks of age. This fact was well illustrated in the present study (see table 4). The economy of feeding an 18% starter ration from 6 to 8 weeks of age is therefore obvious.

Thus, based on the data of this and other experiments conducted in this laboratory, it is recommended that pigs weaned at 2 weeks of age be fed a 24% protein early-weaning ration for only a 4-week period (i.e. to 6 weeks of age), and at that time changed to an 18% starter ration. The latter is in harmony with the findings of Smith and Lucas (1957) who observed no advantage in raising the level of crude protein above 18% for pigs weighing 25–50 lb.

TABLE 5. PHYSICAL COMPOSITION OF A 24% PROTEIN EARLY-WEANING PIG RATION

Ration ingredient	%
Wheat	20
Oat groats	30
Soybean oilmeal	9
Fishmeal	9
Brewers' yeast	5
Skimmilk powder	14
Cane molasses	10
Vitamin-mineral-antibiotic supplement [a]	3

[a] Composition given by Lloyd and Crampton (1958a).

Summary

The correction of apparent essential amino acid imbalances in two early-weaning rations and in an 18% protein starter ration by the addition of appropriate amounts of methionine and isoleucine did not enhance the biological value of such rations for pigs.

A 20% protein early-weaning ration was not adequate for pigs weaned at 2 weeks of age.

The minimum nutritionally satisfactory age for changing pigs from an early-weaning ration to a conventional 18% protein starter ration was 6 weeks of age, and the recommended level of protein in the early-weaning ration was set at 24%.

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EFFECT OF CALCIUM:PHOSPHORUS RATIO, OLEANDOMYCIN AND PROTEIN LEVEL ON THE PERFORMANCE OF EARLY-WEANED PIGS

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THE adverse physiological effect of a high calcium:phosphorus ratio in the diet of various animal species has been known for many years. However, only limited information is available on the effects of excessive intakes of calcium by early-weaned pigs. Using pigs weaned at three weeks of age, Conrad and Beeson (1957) noted that in rations carrying from 0.58% to 0.68% phosphorus a dietary level of 1.06% calcium greatly reduced gains and increased the incidence of parakeratosis as compared to a level of 0.68% calcium.

Recent reports in the literature point to a possible interrelationship between dietary calcium and antibiotics (Conrad and Beeson, 1957; Eggert *et al.*, 1959; Price and Hare, 1959). In addition, various studies have supported the claim that antibiotics, when added to swine rations, have a "protein-sparing" effect (Cunha *et al.*, 1950; Catron *et al.*, 1952; Burnside *et al.*, 1954; Sewell and Keen, 1958). On the other hand, Hoefer *et al.* (1952) and Wallace *et al.* (1959) failed to observe this latter effect.

The objectives of the research to be reported were (1) to examine the effect of excessive intakes of calcium on the performance of early-weaned pigs fed rations containing two levels of protein, and (2) to determine the effect of oleandomycin on the protein requirements of early-weaned pigs and on the stress created by excessive intakes of calcium.

Experimental

A total of 96 pigs, divided equally as to sex, were weaned and placed on test at an average age of 17.5 days in three replicates. Each replicate consisted of 32 pigs fed for a 6-week period. The pigs were allotted at random and penned individually in a suitably heated building.

The relevant composition of the rations fed is given in table 1. The proportions of ingredients used in rations 1–8 were identical to those contained in the early-weaning pig ration developed in this laboratory by Crampton and Ness (1954). In rations 9–16, the same ingredients were used, but their proportions were altered to provide 22% protein rations similar to those fed by Lloyd and Crampton (1958a). The ingredients in the vitamin-mineral supplement were similar to those described by Lloyd and Crampton (1958b).

The high phosphorus level of 1.0% was not chosen as such. This was actually the level of phosphorus supplied by the non-mineral feed ingredients of the original 30% early-weaning ration. Dicalcium phosphate was used to bring the phosphorus level of the 22% protein rations up to 1.0%, and calcium carbonate was used to widen the calcium:phosphorus ratio where applicable. These additions were made at the expense of molasses.

Chromic oxide was incorporated in all rations to the extent of 0.25% of the dry matter content for use in the calculation of digestion coefficients according to the method of Schürch *et al.* (1953).

The pigs were allowed to eat all rations *ad libitum;* water was available at all times. Each pig was weighed at weekly intervals and the weekly feed consumption per pig was also recorded. During the sixth week of each replicate, representative feces samples were collected daily from each pig. These were dried, composited for each pig at the end of the period and ground for subsequent chemical analyses.

Results

The average daily gain, daily feed consumption and daily gain adjusted by regression to the average feed intake (i.e. feed efficiency) of the 96 pigs are summarized according to main treatment comparisons in table 2.

From these data, it is seen that increasing ration calcium resulted in a significant (P=

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TABLE 1. COMPOSITION OF RATIONS

Ration description	30% Protein rations (Ration numbers 1 to 8)	22% Protein rations (Ration numbers 9 to 16)
Ingredients,%		
Wheat	10	20
Oat groats	15	36
Skimmilk powder	37	10
Soybean oilmeal	10	8
Fishmeal	10	8
Brewers' yeast	5	5
Molasses	10	10
Vitamin-mineral supplement	3	3

Ration number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Calcium, %	1.2	1.2	2.0	2.0	3.0	3.0	4.0	4.0	1.2	1.2	2.0	2.0	3.0	3.0	4.0	4.0
Phosphorus, %	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Oleandomycin (10 gm./ton of ration)	Nil	+	Nil	+	Nil	+	Nil	+	Nil	+	Nil	+	Nil	+	Nil	+

0.05) decrease in average daily gains. Although average daily feed consumption was not decreased significantly by raising calcium levels in the ration, it tended to parallel the decrease in average daily gains. When the latter were adjusted to equal feed intake, it was found that ration calcium level had no statistically significant effect on feed efficiency.

The inclusion of oleandomycin in these early-weaning pig rations at a level of 10 gm. per ton resulted in a highly significant (P=0.01) increase in average daily gains, and a significant (P=0.05) increase in average daily feed consumption. When gains were adjusted to equal feed intake, oleandomycin still produced a highly significant (P=0.01) increase. There was no significant interaction between oleandomycin and ration calcium level.

There were no differences in average daily gain, feed consumption or feed efficiency attributable to ration level of protein, nor was the interaction between protein level and oleandomycin significant.

The average apparent digestion coefficients of gross energy, crude protein, ether extract and total carbohydrate for the 96 pigs are summarized according to main treatment comparisons in table 3.

The only effect of increasing ration calcium levels was a numerically small but highly significant (P=0.01) decrease in the apparent digestibility of total carbohydrate.

Oleandomycin brought about a highly significant (P=0.01) increase in the apparent digestibility of gross energy and crude protein. The increase with ether extract was significant only at the 5% level. Total carbohydrate

TABLE 2. AVERAGE DAILY GAIN, DAILY FEED CONSUMPTION AND DAILY GAIN ADJUSTED TO EQUAL FEED INTAKE OF 96 PIGS ACCORDING TO MAIN TREATMENT COMPARISONS

Main treatment comparisons	No. of pigs	Average daily gain	Average daily feed consumption	Average daily gain adjusted*** to equal feed intake
		(lb.)	(lb.)	(lb.)
1.2% Calcium	24	0.70	1.54	0.67
2.0% Calcium	24	0.68	1.49	0.66
3.0% Calcium	24	0.57*	1.33	0.61
4.0% Calcium	24	0.59	1.45	0.60
Oleandomycin—nil	48	0.56	1.37	0.59
Oleandomycin—10 gm./ton	48	0.71**	1.53*	0.68**
30% Protein	48	0.65	1.44	0.65
22% Protein	48	0.63	1.46	0.62

* Statistically significant "F" value (P=0.05).
** Statistically significant "F" value (P=0.01).
*** Statistically adjusted by regression method.

TABLE 3. AVERAGE APPARENT DIGESTION COEFFICIENTS OF CERTAIN RATION COMPONENTS BY 96 PIGS ACCORDING TO MAIN TREATMENT COMPARISONS

Main treatment comparisons	No. of pigs	Average apparent digestion coefficients			
		Gross energy	Crude protein	Ether extract	Total carbohydrate
1.2% Calcium	24	85	84	58	92
2.0% Calcium	24	85	83	62	90
3.0% Calcium	24	85	84	60	89**
4.0% Calcium	24	85	85	65	89
Oleandomycin—nil	48	84	82	58	90
Oleandomycin—10 gm./ton	48	86**	86**	64*	90
30% Protein	48	87	86	60	91
22% Protein	48	84**	82**	62	89

* Statistically significant "F" value (P=0.05).
** Statistically significant "F" value (P=0.01).

digestibility was not affected by the inclusion of oleandomycin.

The apparent digestion coefficients for the gross energy, crude protein and total carbohydrate of the 30% protein rations were highly significantly greater (P=0.01) than those of the 22% protein rations.

Discussion

The deleterious effects of high levels of calcium in early-weaning pig rations were quite mild in this experiment. Despite the relatively large amounts of calcium fed, the observed decrease in average daily gain was significant only at the 5% level, and no real differences in feed intake or feed efficiency were noted. Of the ration components studied, the digestibility of only total carbohydrate was significantly (P=0.01) affected, and it was noted that the reduction was but three percentage units from the lowest to highest calcium level. This is in contrast to the findings of Colovos *et al.* (1958) who indicated that excess calcium in the dairy cattle ration has a depressing effect on the digestibility of both energy and protein.

A closer examination of the effect of ration calcium level on gains revealed that the daily gains made by the pigs on the 1.2% and 2.0% calcium levels were similar while those made by the pigs on the 3% and 4% calcium levels were similar and lower. Thus, under the conditions of this experiment, a critical calcium:phosphorus ratio appeared to exist between 2:1 and 3:1.

This suggested ratio is quite wide, especially when compared to the narrow ratio of 1.5:1 indicated by Bohstedt (1955) for swine. However, there may be a partial explanation for this and for the absence of any pronounced adverse effects in this experiment due to increasing calcium levels. As noted previously, the combination of feed ingredients in the 30% protein ration resulted in a basic phosphorus level of 1.0%, this being well above the requirement of 0.6% for a 10-lb. pig. In addition, the vitamin D level was 8–9 times above the requirement for a pig of this size. Thus, because the optimum calcium:phosphorus ratio varies somewhat according to the level of the individual elements and to the level of vitamin D, the presence of excessive amounts of phosphorus and vitamin D may have resulted in a critical calcium:phosphorus ratio that was wider than might be expected for pigs of this age and size.

It is of interest that even at the high levels of calcium, no symptoms of parakeratosis were noted during the 6-week test period. This is in sharp contrast to earlier work in this field as exemplified by the findings of Lewis *et al.* (1956) who pointed out that pigs fed rations containing 1.1 to 1.4% calcium (phosphorus level at 0.5%) rapidly developed parakeratosis, and by those of Luecke *et al.* (1957) who found that a ration containing 1.25% calcium and 0.95% phosphorus resulted in 100% incidence of parakeratosis. In both of these reports, however, the pigs were older and the test periods were of longer duration than 6 weeks. On the other hand, Conrad and Beeson (1957), feeding early-weaned pigs for periods of 6–10 weeks, observed severe parakeratotic symptoms with rations containing 1.06% calcium and 28 p.p.m. of zinc. Our rations contained no added zinc.

No significant statistical interactions between level of calcium and oleandomycin were noted.

Oleandomycin produced a 25% increase in daily gains and a 15% increase in feed efficiency. These pronounced effects were con-

sistent throughout the three replicates; they were attributable, at least in part, to significant increases in the digestibility of crude protein and ether extract, and consequently of gross energy. Beneficial effects from this antibiotic have been noted by others. Biely and March (1959) obtained significant growth promotion when oleandomycin was included in the chick ration. Working with pigs, Hawbaker *et al.* (1960) observed improvements in both growth rate and feed efficiency when oleandomycin was added to the ration, maximum responses being obtained with baby pigs.

Although the apparent digestibility of the gross energy, crude protein and total carbohydrate of the 30% protein rations was significantly higher than that of the 22% protein rations, the latter produced gains equal in magnitude and efficiency to those produced by the former. The adequacy of this lowered protein level in early-weaning pig rations is in agreement with recent findings (Jensen *et al.*, 1957; Peo *et al.*, 1957; Lloyd and Crampton, 1958a).

Summary

Increasing calcium from 1.2% to 4.0% in early-weaning pig rations significantly decreased average daily gain and the apparent digestibility of total carbohydrate, but had no other adverse effects.

The inclusion of oleandomycin in early-weaning pig rations resulted in a significant increase in average daily gain, average daily feed consumption and feed efficiency. The apparent digestibility of the gross energy, crude protein and ether extract of rations containing this antibiotic was significantly increased.

The apparent digestibility of gross energy, crude protein and total carbohydrate was significantly higher in 30% protein rations than in 22% protein rations; however, gains were equal in magnitude and efficiency.

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EFFECT OF REPEATED INJECTIONS OF IRON-DEXTRAN UPON BLOOD HEMOGLOBIN AND HEMATOCRIT AND UPON THE IRON AND MYOGLOBIN CONCENTRATION AND COLOR OF THE *SEMIMEMBRANOSUS* MUSCLE OF SWINE [1]

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A COMMON practice for the treatment and prevention of anemia in swine is the administration of iron either orally or parenterally. Intramuscular injection of iron-dextran has been shown by Ullrey *et al.* (1959), among others, to increase the hemoglobin levels of baby pigs. Bray *et al.* (1959) found that oral administration of iron and copper significantly increased the myoglobin content of veal muscle. This was accompanied by a darkening of the surface color. In a study conducted with swine, Henry and Bratzler (1960) found that supplementation of the diet with iron had no significant effect upon muscle color or myoglobin concentration in the *Longissimus dorsi* muscle.

This study was conducted to observe the effects of repeated large intramuscular injections of iron-dextran on swine from birth to market weights.

Experimental

Sixteen crossbred barrows and gilts were used in this study. They received initially 100 mg. of iron, as iron-dextran,[3] injected in the right ham at 3 days of age to prevent baby pig anemia. Subsequent injections of iron-dextran into the experimental animals were made as nearly as possible into the *semimembranosus* of the left ham according to the schedule shown in table 1.

Two control and two experimental animals were slaughtered at 7, 9, 16 and 22 weeks of age; they averaged 20, 40, 120, and 180 lb., respectively, in body weight. The animals were slaughtered 2–4 weeks after the last injection. Blood hemoglobin and hematocrit determinations were made on the day prior to slaughter. The carcasses were chilled at 36–40° F. for a period of 2 days. The *semimembranosus* muscles were removed from both hams of the chilled carcasses for determinations of myoglobin and surface color by methods previously described (Henry and Bratzler, 1960). Total iron determinations were made on a core 1-inch in diameter taken from the *semimembranosus* muscles from the pigs slaughtered at 180 lb. The method used was that of Bandemer and Schaible (1944) as modified by Ullrey *et al.* (1960).

TABLE 1. SLAUGHTER AGE AND WEIGHT OF PIGS, AND TREATMENT

Group no.[a]	Slaughter age, weeks	Average slaughter wt., lb.	Treatment[b] (iron-dextran)
1	7	30	Experimental pigs received 150 mg. iron at 5 weeks of age.
2	9	40	Experimental pigs received 200 mg. iron at 5 weeks and 250 mg. at 7 weeks of age.
3	16	120	Experimental pigs received 200 mg. iron at 5 weeks, 250 mg. at 7 weeks, 300 mg. at 9 weeks and 350 mg. at 11 weeks of age.
4	22	180	Experimental pigs received 150 mg. iron at 5 weeks, 200 mg. at 7 weeks, 250 mg. at 9 weeks, 300 mg. at 11 weeks, 350 mg. at 13 weeks and 400 mg. at 17 weeks of age.

[a] Each group consisted of two control and two experimental animals.
[b] Each injection was in the *semimembranosus* of the left ham.

Results and Discussion

Table 2 summarizes the results of analysis of the blood and muscles of the experimental

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[2] The authors wish to acknowledge the assistance of C. L. Zutaut in performing the muscle iron determinations.
[3] Armidexan-75, manufactured by Armour and Company, Kankakee, Illinois.

and control animals. It may be noted that the animals at 16 and 22 weeks of age receiving repeated injections of iron-dextran had consistently lower hematocrits and hemoglobin levels than the control pigs. Numerous workers have found that baby pigs receiving intramuscular injections of iron-dextran or oral iron preparations had increased hemoglobin levels at an early age. Miller *et al.* (1959) made the further observation that continued feeding of high levels of iron failed to increase further the hemoglobin level in older animals.

within treatments as well as between treatments. However, surface color varied with myoglobin concentration. In general, as myoglobin increased, the surface color became darker. Color indices were computed from the pork color standard as previously described (Henry and Bratzler, 1960). The negative numbers (table 2) indicate that the samples were darker than the standard, and the positive numbers, lighter. At the early slaughter ages muscle color of the experimental pigs was somewhat darker than that of the con-

TABLE 2. HEMATOLOGY AND MUSCLE ANALYSIS OF EXPERIMENTAL AND CONTROL ANIMALS

			Muscle analysis					
	Hematology		Myoglobin mg./gm.[a]		Color index[b]		Muscle iron, mcg./gm.[c]	
Group	Hb, gm./ 100 ml.	PCV, %	L. ham	R. ham	L. ham	R. ham	L. ham	R. ham
1								
Control	10.7	36.6	0.830	0.810	4.88	5.60	....	.....
Experimental	11.6	38.2	.905	.854	2.71	1.63	....	.....
2								
Control	9.6	34.1	.793	.793	3.09	3.30	....	.....
Experimental	10.6	36.1	.821	.793	0.48	0.30	....	.....
3								
Control	13.5	39.6	.771	.808	1.01	0.48	....	.....
Experimental	12.3	37.8	.806	.856	−3.10	−3.36	....	.....
4								
Control	15.2	42.8	1.148	1.213	−8.30	−8.61	7.62	9.00
Experimental	13.3	39.8	1.050	1.138	1.63	2.86	9.75	10.40

[a] Myoglobin expressed on a fresh tissue basis.
[b] Represents units from standard. Negative numbers are darker than standard; positive numbers are lighter than the standard.
[c] Muscle iron expressed on a fresh tissue basis.

Undoubtedly there are physiological limitations to the increase in blood hemoglobin and hematocrit resulting from iron administration. The decrease noted in this study remains unexplained.

Although the myoglobin concentration of the *semimembranosus* muscle was higher in the 22-week-old pigs than in the younger animals, there was no apparent relationship to treatment. The increased myoglobin concentration can be attributed to age and maturity. In this study the greatest increase in myoglobin occurred between 16 and 22 weeks of age or between 120 and 180 lb. A highly significant correlation ($r=0.75$) was found between hemoglobin level and myoglobin concentration.

Muscle color, as measured by Munsell spinning disks, indicated large variations

trols. However, in the 22-week-old group, muscle color of the experimental animals was lighter than that of the controls. At no slaughter age was the injected ham significantly darker than the non-injected ham of the same animal.

Iron concentration was somewhat higher in the hams of the experimental animals slaughtered at 22 weeks, but the injected hams contained, on the average, less iron than the non-injected hams. No residual iron was visible in groups slaughtered at 30, 40, and 120 lb.; however, there was a slight rust coloring of the external fat of the hams of animals receiving repeated large doses of iron-dextran. This was apparent only in the 22-week-old animals that had received 6 injections totaling 1650 mg. of iron.

Summary

A study was conducted to determine the effect of repeated intra-muscular injections of iron-dextran in swine. Two experimental and two control animals were slaughtered at 7, 9, 16 and 22 weeks of age, the experimental animals having received a total of 150, 450, 1100 and 1650 mg. of iron, respectively. Hemoglobin and hematocrits increased with repeated injections through 9 weeks of age. At 16 and 22 weeks of age the injected pigs had lower hemoglobin and hematocrits than the control pigs. Repeated injections of iron apparently did not affect the myoglobin concentration or surface color of the *semimembranosus* muscle. However, the external ham fat of animals that had received 1650 mg. of iron had a slight rusty color. Muscle iron concentration was significantly higher in the experimental animals than in the controls at 22 weeks of age; however, the injected ham contained, on the average, less total iron than the non-injected ham.

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GENETIC AND ENVIRONMENTAL INFLUENCES ON GAIN OF BEEF CATTLE DURING VARIOUS PERIODS OF LIFE [1]

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INCREASED rate of gain is a desirable attribute in beef cattle. The consequences of selecting for gain in different periods of the animal's life can be understood best by knowing the relative importance of heredity and environment in different periods and the genetic relationships among the periods. Certain environmental sources of variation usually can be identified and eliminated, at least partially, thus increasing the fraction of the differences among animals which is genetic.

In this study the effects of several environmental variables on beef cattle gains are evaluated and used to adjust the data. Paternal half-sib estimates of heritability and the phenotypic, genetic and environmental correlations among birth weight, weaning weight and gain in five successive 28-day periods of a post-weaning feedlot test were computed.

Source of Data

The data were taken from the records of a herd of purebred Hereford cattle owned by Charles E. Haigler and located near Washington Court House in southern Ohio.

The cows are bred to calve starting the first week in April and most of the calves are born during April and May. Cows and calves are grazed on permanent bluegrass or rotation pastures of alfalfa and bromegrass or alfalfa and timothy without supplemental feeding during the summer and fall. All calves are weaned on the same day each year. The weaning date varied from late November to early January for the years included in this study. A few late summer calves are born each year. These calves are not started on the feeding test and were omitted from this study. The average age at weaning of the calves used in this study was 230 days.

Immediately following weaning all calves are started on a feeding test of 140 days. No selection is practiced prior to the termination of the feeding period. During the post-weaning feeding period the calves are separated by sex, the bulls being fed in one lot and the heifers in an adjoining lot. Concentrates are feed free choice in self-feeders within the pens. The calves are fed as much hay as they will "clean up" each day and are given a small amount of grass silage.

The concentrates fed to the calves are coarsely ground corn and cob meal containing 8 to 9% soybean oil meal. The hay and grass silage are either mixed alfalfa and bromegrass or alfalfa and timothy.

Since the birth weights were missing for 39 calves, weaning weight was used in this study instead of gain from birth to weaning. Gain for each 28-day period during the feeding test was computed. Since weaning weights were not taken in 1952, weaning weight and gain in the first 28-day period were inseparable. Hence, only birth weight and gain in the last four feeding periods could be included in the analysis for the 1952 calves.

In addition to birth weight, weaning weight and gain in each of five 28-day periods after weaning, the following information was available for each calf. Age at weaning (thus age at the beginning of the test) was computed for each calf since the weaning date for each year and the birth date for each calf were known. The age of the dam of each calf was available. Year of birth, sex, sire, and dam of each calf were also recorded.

Birth weights were taken on 793 calves. Weaning weight and gain in the first period were available for only 748 of the calves since the 84 calves born in 1952 were not weighed at weaning. A calf was omitted from the study if any trait was missing except birth weight in any year, or weaning weight and first period gain in 1952. The calves were the progeny of 23 sires.

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Results and Discussion

Table 1 contains the unadjusted means and standard deviations of the seven traits. Since information was available on several sources of variation which are known to affect rate of gain in beef cattle, the data could be adjusted for these variables before proceeding with the the paternal half-sib analyses of variance and covariance. This method has been described by Henderson (1953).

Because the subclass numbers were unequal and some sources of variation could be considered fixed and others random, least squares analysis was used to compute the effects of the known environmental variables. The assumptions necessary for using least squares methods, the mechanics of computation and the characteristics of the estimates have been described by Kempthorne (1952).

TABLE 1. MEANS AND STANDARD DEVIATIONS OF THE UNADJUSTED DATA

Trait	No. of calves	Mean	Standard deviation
Birth weight	793	72	10
Weaning weight	748	431	86
First period gain	748	30	23
Second period gain	832	50	18
Third period gain	832	58	20
Fourth period gain	832	54	20
Fifth period gain	832	55	19

Few studies appear in the literature on the interactions among the environmental variables studied here. In mimeograph form, Landblom (1954) reported tests of significance based on an approximate method about the three two-factor interactions and the three-factor interaction involving sex, age of the dam and year, for weaning weight. In her study only the interaction of sex and age of the dam yielded a significant F value. Dahmen and Bogart (1952) found an exceedingly small interaction between sex and year for post-weaning daily gain.

The methods employed in this study for evaluating the interactions were approximate, as is often necessary when applying statistics to data on large animals. Graphing the two-factor interactions showed that the interaction of year and sex was the least important interaction for all the traits. As most of the other interactions were not important, the interaction of year and sex was not considered further. The higher order interactions were not examined. These seemed unlikely to be important in view of the generally small two-factor interactions.

These data support most other studies in that the effect of age at weaning on weaning weight is nearly linear (figure 1). From these data it also appears that the effect of age at weaning on post-weaning gain was nearly linear. The effect of age of calf on weaning weight and post-weaning gain was treated as linear in later analyses.

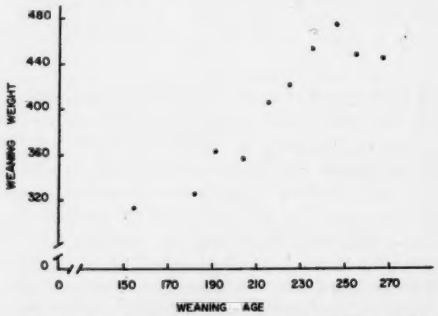

Figure 1. Mean unadjusted weaning weight for each age of calf group.

The effect of age of dam on birth weight and weaning weight in these data is curvilinear. Figure 2 shows the average weaning weight for each age of dam group. Koch's (1955) data provided a good example of this. Blackwell and Henderson (1955) fitted a quadratic equation for the effect of age of dam on weaning weight of lambs.

By treating age of the calf at weaning and age of dam as continuous variables it was possible to make approximate tests of significance about all the two-factor interactions except the

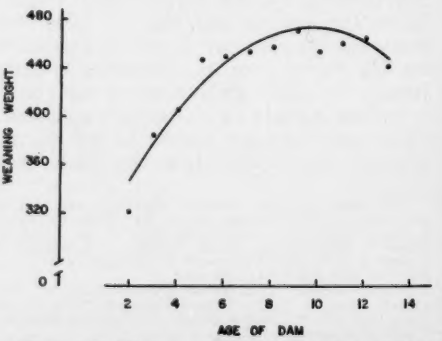

Figure 2. Mean unadjusted weaning weight for each age of dam group and the computed quadratic regression.

interaction between year and sex. It would be desirable to know what fraction of the total sum of squares could be attributed to each interaction; however, this would have required solving several large sets of simultaneous equations.

The general procedure in testing the interactions was to investigate whether a single regression with the independent variable treated as a continuous variable described the data about as well as a separate regression for each level of the discrete variable. For the interaction between age of calf and age of dam, age of dam was treated as the discrete variable.

For each test, all other sources of variation were ignored and their real effects went into the residual sum of squares (if the ignored variables were uncorrelated with the variables being tested). This tends to inflate the residual mean square and to make the F ratio smaller than it really should be. This method makes the number of degrees of freedom for the residual mean square slightly higher than it would have been if all the recognized sources of variation were considered simultaneously. With several hundred degrees of freedom available for estimating the residual mean square, this discrepancy was of minor importance.

As an illustration of the general procedure, an example using year and age of dam follows. The full model allowing a regression for each year was $y_{ij}=b_{0i}+b_{1i}\,x_{ij}+b_{2i}\,x_{ij}^2+e_{ij}$ where y is the trait for the jth calf in the ith year and x is the age of the dam for that calf. The b's are zero unless the i subscript is the same as that of the calf.

The reduced model allows for only one regression representing all years. Since yearly means of the y's and x's were usually different, an intercept was fitted for each year. Otherwise, even if the curves were identical in shape for each year, the differences between the two models could be significant if the yearly intercepts varied much. The reduced model was $y_{ij}=b_{0i}+b_1\,x_{ij}+b_2\,x_{ij}^2+e_{ij}$. A reduction due to each model was computed and a test of significance was made in the ordinary way.

TABLE 2. SAMPLE ANALYSIS OF VARIANCE FOR TESTING THE INTERACTIONS

Source of variation	Degrees of freedom	Mean square
$R(b_{0i} \ldots b_{2i})$	p	
$R(b_{0i} \ldots b_2)$	q	
Difference	p−q	A
Residual	n−p	B
	F p−q, n−p=A/B	

Table 2 shows a sample analysis of variance where p is the number of parameters in the full model, q is the number in the reduced model and n is the total number of observations. The results of testing the interactions for significance are given in table 3. As expected, the interaction between age of calf and sex was important for weaning weight. This means the bulls and heifers grew at different rates up to weaning. Koch *et al.* (1959) found a difference of 0.11 lb. between the average daily gain of bulls and heifers from birth to weaning. This interaction was also significant for the gain in the last period. Since this interaction was not consistently important for the other post-weaning periods, separate regressions for each sex were not used for adjusting those gains.

The interaction between age of dam and year was consistently important. Further investigation of this interaction showed that it was largely due to a change in policy from two- to three-year old calving beginning in 1956. To allow for this, separate equations were fitted for three-year old dams having first and second calves.

TABLE 3. RESULTS OF TESTS OF SIGNIFICANCE OF THE TWO-FACTOR INTERACTIONS BETWEEN THE ENVIRONMENTAL EFFECTS

	Birth weight	Weaning weight	Gain in period				
			One	Two	Three	Four	Five
Age of calf x year	..	NS[a]	NS	NS	NS	NS	NS
Age of calf x sex	..	**[b]	NS	NS	NS	NS	**
Age of calf x age of dam	..	NS	NS	NS	NS	NS	NS
Age of dam x year	NS	**	*[c]	NS	**	NS	**
Age of dam x sex	NS	NS	NS	NS	*	NS	NS

[a] NS P>0.05.
[b] ** P<0.01.
[c] * 0.05>P>0.01.

Figure 2 shows the mean weaning weight for each age of dam group and the quadratic regressions fitted from the data. The curve would fit the data better if it rose more steeply for young cows and fell more slowly for older cows (birth weight was similar). A quadratic curve must be symmetrical around a line parallel to the y-axis. Therefore, age of dam was treated as a discrete variable in this analysis, since it was felt that the quadratic regression did not represent adequately the biology of the aging process.

for any trait were biased for the older ages if cows remained in the herd because they were genetically better for that trait.

The age of dam constants show there was a tendency for the calves from younger and older cows, which probably give less milk on the average than the middle-aged cows, to gain more in the first post-weaning period. This initial advantage in post-weaning average daily gain of the calves from poorer milkers is overcome in the second and third periods and reversed toward the end of the

TABLE 4. ENVIRONMENTAL EFFECTS COMPUTED FROM THE LEAST SQUARES ANALYSIS

Year	Birth weight	Weaning weight	Gain in period				
			One	Two	Three	Four	Five
1950	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1951	—4.3	17.0	—13.7	11.9	16.5	12.4	—3.9
1952	—2.0	....	....	2.7	30.0	31.3	19.3
1953	—1.5	17.1	—12.1	9.2	32.6	24.4	27.2
1954	—7.3	32.5	—13.3	15.1	18.1	17.9	19.5
1955	0.2	26.0	—29.2	7.7	21.1	24.0	6.5
1956	—3.2	1.7	—44.9	—6.4	17.1	30.5	16.7
1957	—4.8	—5.2	—21.1	—2.3	17.1	15.7	15.2
1958	—1.2	10.0	—40.4	—0.9	37.8	33.4	9.8
Age of dam							
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	2.3	...	...	...	...	...	...
3 (1st calf)	...	61.1	0.8	2.7	11.9	4.4	20.2
3 (2nd calf)	...	19.4	—2.2	—2.0	2.6	5.9	8.2
4	7.6	44.8	—5.4	—3.5	8.0	7.0	8.6
5–7	9.5	93.3	—0.6	3.0	9.5	7.0	10.6
8–12	9.5	102.6	—0.8	3.0	8.9	8.0	11.0
13–17	4.8	83.8	1.6	0.7	10.9	2.5	13.1
Sex							
Bulls	3.9	45.4	2.2	8.4	8.8	11.3	10.4
Heifers	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Age of calf							
Bulls	...	2.0	...	...	...	...	...
Heifers	...	1.4	...	...	...	...	...
Bulls and heifers	...	...	0.11	0.08	0.04	0.02	0.01

An additive model was fitted in order to adjust post-weaning gains for the environmental effects. This model included years, sexes, ages of dam, sires and a linear regression for age of calf. For weaning weight a different linear regression was included in the model for each sex. For birth weight this regression was omitted from the model.

The constants for years, sexes, ages of dam and the age of calf regressions obtained from the above analysis are shown in table 4. The data were adjusted using these constants. Accuracy for this method requires that no interactions existed between sires and the other variables and that no correlations existed among the variables. The age of dam constants

test. This suggests that the net effect of age of dam on post-weaning gains depends on length of the feeding period and does not necessarily diminish after a few weeks. Since this is contrary to popular opinion further study is warranted. It does appear that taking into account the age of the dam permits more accurate evaluation of animals for post-weaning gain.

The average daily gain from birth to weaning was 1.61 lb. for bulls and 1.46 lb. for heifers. The regressions of weight on age computed from the least squares analysis were 2.0 lb. for the bulls and 1.4 lb. for the heifers. This difference indicates that the bulls grew at a much faster rate immediately prior to wean-

TABLE 5. EXPECTED MEAN SQUARES AND MEAN PRODUCTS FOR THE PATERNAL HALF-SIB ANALYSES

Source of variation	Degrees of freedom	Expected mean square	Expected mean product
Sires	22	$\sigma_A^2+k\sigma_S^2$	cov (A_i, A_j)+k cov (S_i, S_j)
Half sibs	$\Sigma(n_i-1)$	σ_A^2	cov (A_i, A_j)

ing than they did earlier. The sum of the regressions of post-weaning gains on age at weaning over the 140-day period was .26 lb. of total gain for each day deviation from average. The effect is not large as this would amount to only 0.1 lb. average daily gain for a deviation of 56 days in weaning age. For a period shorter than 140 days the effect on average daily gain would be larger because the regression of gain on initial age of calf was larger for the earlier periods.

The differences between bulls and heifers are in good agreement with those reported in other studies. The average sex difference for post-weaning gains was 0.3 lb. gain per day.

A paternal half-sib analysis was made on the adjusted data. The analyses of variance and covariance (Hazel *et al.*, 1943) are illustrated in table 5. No allowance was made for the degrees of freedom used in computing the environmental constants from the data. Since these were not distributed exactly proportionately among the sires, the components of variance and covariance were biased to some extent (Henderson, 1953). This bias would be small.

The model assumed is $P_i=G_i+E_i$ where P_i is the phenotype, adjusted for the environmental factors, G_i is the sum of the average effects of the genes affecting the phenotype and E_i is the environmental contribution to the phenotype. If the sire differences are entirely genic, the necessary variances may be estimated as:

$$\sigma_G^2=4\sigma_S^2$$
$$\sigma_E^2=\sigma_A^2-3\sigma_S^2$$

where σ_A^2 is the component of variance for half-sibs and σ_S^2 is the component for sires, and similarly for the covariances. The necessary assumptions are random mating, no epistatic effects, no environmental correlations among a sire's progeny and no covariance between genotype and environment. Multiplication of the component for sires by four assumes the average relationship among the half-sibs was one-fourth. This relationship would be slightly higher in this herd, because related groups of cows were usually mated to unrelated bulls. However, it seems unlikely to be higher than 0.28. This would bias the genic variances and covariances slightly upward. Heritabilities (table 6) and the phenotypic, genetic and environmental correlations (table 7) were computed from these variances and covariances. Correlations computed from components can exceed unity due to sampling error. Using Robertson's (1959) formula, the standard errors of the genetic correlations are approximately 0.3.

The heritabilities for birth weight, weaning weight, post-weaning gain and final weight are in fairly good agreement with estimates for these traits from other studies. Considering the separate periods, heritability was highest for the second 28-day period and decreased for successive periods. This indicates that environmental differences become more important as the feeding period progresses.

In general, the genetic correlations are large, the environmental ones low or negative and the phenotypic ones intermediate. The genetic correlations tended to be higher between gains made in adjacent time periods and to be lower as the periods became more remote. The environmental correlations of weaning weight with post-weaning gains indicate that good pre-weaning environment is a handicap to early post weaning gains but enhances later gains. This was similar to what was shown by the age of dam constants. Negative environmental correlations exist between adjacent periods. Errors in weighing, differences in fill

TABLE 6. HERITABILITIES AND THEIR APPROXIMATE STANDARD ERRORS

Trait	Symbol	Heritability	Standard error
Birth weight	P_1	0.22	0.10
Weaning weight	P_2	.25	.11
First period gain	P_3	.18	.09
Second period gain	P_4	.28	.11
Third period gain	P_5	.18	.08
Fourth period gain	P_6	.08	.06
Fifth period gain	P_7	.04	.05
140-day feedlot gain	P_8	.40	.14
Final weight	P_9	.47	.16

TABLE 7. PHENOTYPIC, GENETIC AND ENVIRONMENTAL CORRELATIONS

		P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9
	Phen.	.31	.14	.14	.11	.15	.11	.26	.36
P_1	Gen.	.69	.94	1.04	.12	1.03	.12	.85	.78
	Env.	.19	—.05	—.16	.10	.02	.11	.01	.18
	Phen.	...	.11	.22	.11	.14	.02	.24	.87
P_2	Gen.	...	1.21	.93	.62	.75	—.25	.93	.98
	Env.	...	—.19	—.04	—.02	.03	.05	—.08	.84
	Phen.	...	...	.06	—.01	.01	.01	.47	.32
P_3	Gen.	...	...	1.06	.51	.74	.27	.94	1.10
	Env.	...	...	—.22	—.12	—.09	—.02	.31	.00
	Phen.	...	...	...	.02	.08	.03	.48	.41
P_4	Gen.	...	...	...	.44	.87	.19	.93	.95
	Env.	...	...	...	—.10	—.06	.02	.26	.11
	Phen.	...	...	...	...	.06	.17	.48	.33
P_5	Gen.	...	...	...	...	.88	.24	.74	.69
	Env.	...	...	...	...	—.06	.17	.41	.20
	Phen.	...	...	...	...	...	.19	.53	.37
P_6	Gen.	...	...	...	...	...	.82	1.00	.88
	Env.	...	...	...	...	...	.15	.45	.27
	Phen.	...	...	...	...	...	...	.54	.29
P_7	Gen.	...	...	...	...	...	...	.46	.08
	Env.	...	...	...	...	...	...	.63	.39
	Phen.	...	...	...	...	...	...	...	.69
P_8	Gen.	...	...	...	...	...	...	...	.98
	Env.	...	...	...	...	...	...	...	.47

at the end of the different periods, and compensatory gain could contribute to these.

Summary and Conclusions

The effects of sex, year, age of dam and age of calf at weaning were evaluated for gain in Hereford bull and heifer calves during different time periods up to one year of age. The least squares constants for the effects of age of dam and weaning age indicate that age of dam and perhaps weaning age should be considered in evaluating calves for post-weaning gains. These data suggest that different regressions of weaning weight on age of calf should be used for bulls and heifers to adjust weaning weight for age at weaning.

Heritability was 0.25 for weaning weight, 0.40 for 140-day feedlot gain and 0.47 for weight at a year of age. These estimates agree reasonably well with previous estimates in the literature. They indicate that considerable progress should result from selection for rate of gain. If the relatively high heritabilities of early feedlot gains as compared with those made later during the post-weaning period should be verified in later studies, measurement of gaining ability can be made at relatively young ages. The large genetic correlations between gains made in different periods suggest that the same genes are largely responsible for gains made during the first year.

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OPTIMUM LENGTH OF FEEDING PERIOD IN SELECTING FOR GAIN OF BEEF CATTLE [1]

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THE ability to gain weight rapidly is one of several valuable economic traits in beef cattle. Increased rate of gain has definite economic value because of its association with economy of gain and because many of the costs of production are on a per head or per unit of time basis. The cost of evaluating this trait would be reduced if the post-weaning evalaution period could be shortened with little loss of information about the breeding values of the animals.

The data used in this study have been described in an earlier paper (Swiger, 1961). That paper presented an evaluation of the effects of year, sex, and age of the dam on birth weight, and of these variables plus age of the calf at weaning on weaning weight and gain in each of five successive 28-day periods following weaning. A high concentrate ration was fed free choice for 140 days beginning immediately after weaning. The data were taken from 832 Hereford calves from an Ohio purebred herd over a period of 9 years (1950–1958). The calves were the progeny of 23 sires. Heritability was estimated for birth weight, weaning weight and gain in each of the five post-weaning periods after adjusting for the environmental factors mentioned above. The genetic, environmental and phenotypic correlations among the traits were also estimated.

In the present paper the effect of shortening the post-weaning feeding test on the accuracy of selecting for weight at one year of age is studied. Indexes are computed which maximize the correlation between hereditary growth and final weight, feedlot gain and weaning weight using various combinations of the traits. The selection differentials are examined for final weight resulting from culling some calves at weaning with later selection of survivors after a test period.

Results and Discussion

The statistical techniques were similar to those used by Hazel *et al.* (1943) with swine data which were later applied to beef cattle by Knapp and Clark (1947) and Urick *et al.* (1957).

The assumption is made that $P_i = G_i + E_i$ where P_i is the adjusted phenotype, E_i is the environmental contribution and G_i is the sum of the average effects of the genes for gain during the ith time period. Then $G_t = \Sigma G_i$ is the sum of the average effects of the genes for final weight. The G_t may be predicted by an index (I) based on one or more phenotypic traits (P_i) containing information about G_t. The index is the multiple regression equation $I = \Sigma b_i P_i$, where the b_i's are chosen to maximize $r_{G_t I}$. The same b_i's result from minimizing $\Sigma(G_t - I)^2$ and are obtained by solving the following equations:

$$b_1 \sigma_{P_1}^2 + b_2 \operatorname{cov} P_1 P_2 + \ldots = \operatorname{cov} P_1 G_t$$
$$b_1 \operatorname{cov} P_1 P_2 + b_2 \sigma_{P_2}^2 + \ldots = \operatorname{cov} P_2 G_t$$
$$\vdots$$

The phenotypic and genic variances and covariances needed to solve the least squares equations are given in table 1. These were computed from the paternal half-sib analysis described in an earlier paper (Swiger, 1961).

The change in the genic effects for final weight which is achieved by selection on an index is proportional to $r_{G_t I}$. Therefore, alternative indexes can be compared on the basis of their correlations with G_t. Since cov $(G_t, I) = \sigma_I^2$ this correlation reduces to σ_I / σ_{G_t}.

P_1 is the adjusted phenotype for birth weight, P_2 for weaning weight, P_3 to P_7 the gains made in five post-weaning periods, and similarly for G_1 to G_7. Then $G_t = \sum_{i=2}^{7} G_i$, since final weight is weaning weight plus gain in all

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TABLE 1. PHENOTYPIC AND GENIC VARIANCES AND COVARIANCES

		P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9
P_1	Phen.	80	164	23	20	15	22	15	95	260
	Gen.	17	86	30	37	3	20	1	93	179
P_2	Phen.	..	3570	115	214	106	132	21	588	4158
	Gen.	..	899	277	241	125	107	—22	727	1626
P_3	Phen.	..	..	330	18	—2	3	1	350	465
	Gen.	..	..	58	70	26	27	6	186	463
P_4	Phen.	..	..	..	269	6	23	8	324	538
	Gen.	..	..	..	74	26	36	5	209	450
P_5	Phen.	..	..	..	..	256	15	42	317	422
	Gen.	..	..	..	..	45	28	5	129	254
P_6	Phen.	..	..	..	..	..	269	49	358	490
	Gen.	..	..	..	..	..	22	11	124	231
P_7	Phen.	..	..	..	..	..	..	243	343	363
	Gen.	..	..	..	..	..	..	9	35	13
P_8	Phen.	..	..	..	..	..	..	..	1692	2280
	Gen.	..	..	..	..	..	..	..	685	1412
P_9	Phen.	..	..	..	..	..	..	..	..	6438
	Gen.	..	..	..	..	..	..	..	..	3038

five periods. The standard deviation of G_t was 55.1.

The regression of G_t on each of the seven traits and the correlation between each trait and G_t are shown in table 2 (I_1 to I_7). Weaning weight and gain in the first two periods contained the most information about the genic effects for final weight.

In order to investigate the effect of shortening the feeding period, six indexes (I_8 to I_{13}) were computed by including successively the traits as they would be measured in a testing program. These indexes and their correlations are also shown in table 2. While including each additional trait as it can be measured increased the correlation, gain in the last two periods added little information, increasing the correlation only from 0.80 to 0.82 (I_{11} and I_{13}). Gain in the fourth period increased the correlation only a little because it largely repeated information given by earlier periods, as indicated by its high genic covariances with earlier periods. Gain in the last period (fifth month on test) was influenced largely by nongenetic factors. The negative coefficient for last period gain in I_{13} suggests that the geneti-

TABLE 2. REGRESSION COEFFICIENTS FOR THE INDEXES PREDICTING THE GENIC EFFECTS FOR FINAL WEIGHT (G_t) AND THE CORRELATIONS BETWEEN G_t AND EACH INDEX

Index	P_1	P_2	P_3	P_4	P_5	P_6	P_7	σ_I	r_{G_tI}
I_1	2.23	....	....	....	....	....	....	....	.36
I_2	....	.46	....	....	....	....	....	....	.49
I_3	....	....	1.40	....	....	....	....	....	.46
I_4	....	....	....	1.67	....	....	....	....	.50
I_5	....	....	....	....	.99	....	....	....	.29
I_6	....	....	....	....	....	.86	....	....	.26
I_7	....	....	....	....	....	....	.05	....	.02
I_8	1.43	.39	....	....	....	....	....	29.8	.54
I_9	1.03	.37	1.17	....	....	....	....	36.4	.66
I_{10}	.96	.30	1.16	1.29	....	....	....	42.1	.76
I_{11}	.84	.28	1.18	1.29	.81	....	....	44.0	.80
I_{12}	.73	.27	1.19	1.27	.79	.51	....	44.8	.81
I_{13}	.77	.26	1.19	1.27	.84	.56	—.32	45.0	.82
I_{14}	....	.41	1.26	....	....	....	....	35.3	.64
I_{15}	....	.34	1.22	1.32	....	....	....	41.3	.75
I_{16}	....	.31	1.23	1.33	.84	....	....	43.4	.79
I_{17}	....	.29	1.23	1.29	.82	.55	....	44.3	.80
I_{18}	....	.29	1.23	1.30	.86	.60	—.29	44.5	.81

cally superior calves gained poorly during the last 28 days. This is indicated by the negative genic covariance between weaning weight and last period gain. While this result is not impossible, sampling error seems more probably the responsible factor.

Five indexes (I_{14} to I_{18}) were computed successively adding the traits as before but ignoring birth weight. This was done to see if birth weight could be omitted with little loss of accuracy in predicting G_t (table 2).

While birth weight alone was a good predictor of the genic effects for final weight, it added little if later information was available. The only appreciable increase in the correlation was when birth weight and weaning weight were combined, as compared with knowing weaning weight only. This increased the correlation from 0.49 to 0.54 (I_2 and I_8). Selection is not customarily practiced at birth in beef cattle. From the results of this study it does not seem advisable to use birth weight to predict genic effects for 12-month weight if weights at weaning and later are taken. If birth weight can be taken rather economically and a post-weaning test is not part of the testing program, it may be worth while.

Omitting birth weight and gain in the last two periods decreased the correlation slightly from 0.82 to 0.79 (I_{13} and I_{16}). An index using weaning weight and aggregate gain in the first 84 days of the feeding period [$I_{19}=0.32\ P_2+1.15\ (P_3+P_4+P_5)$] instead of gain in each period (I_{16}) was computed. The correlation between I_{19} and G_t was 0.78. Therefore, I_{19} was $0.78/0.82=0.95$ as efficient for selection as I_{13} which combined all traits in the most efficient manner. Considering the cost of feeding for the last 56 days, I_{19} seems to be a logical index to use. If the calves are to be kept on a full feed following an 84-day evaluation period in order to fatten or develop them for sale, the additional information required for I_{13} can be utilized with little extra cost or trouble except for taking the weights. Standardizing the regression coefficients shows that, for I_{19}, weaning weight is 0.86 as important as 84-day gain in determining the differences in the index values.

Morley (1950) gave the genetic increase in each trait expected per unit of I as $\sum_j b_j \text{ cov } (G_i, G_j)/\sigma_I^2$. For I_{19} these are 0.06, 0.55, 0.14, 0.14, 0.09, 0.07 and 0.01 lb. respectively, for the seven periods. The sum of the last six (omitting birth weight) equals unity since the increase for birth weight is also included in the increase for weaning weight. The expected increase in preweaning gain would be 0.49 lb.

Several indexes were computed predicting the genic effects for feedlot gain where $G_t'=\sum_{i=3}^{7} G_i$. G_t' would be of primary interest to a feeder calf buyer. The standard deviation of G_t' was 26.2. The regressions of G_t' on each trait and the correlations are shown in table 3 (I_1' to I_7'). The combination of birth weight and weaning weight provide a fairly accurate indication of feedlot gain (I_8'). Comparing I_9' and I_{10}' shows that 84-day gain was 0.94 as accurate for predicting the genic effects for 140-day gain as was 140-day gain.

If a breeder is selling calves at weaning, the correlation of the genic effects for weaning weight (G_2) and an index predicting G_2 using weaning weight above or a combination of birth weight and weaning weight would be of interest. These indexes turn out to be:

$I_1''=0.25\ P_2$, where $r_{G_2I_1''}=0.50$ and

$I_2''=0.61\ P_1+0.22P_2$, where $r_{G_2I_2''}=0.53$

Hence birth weight adds very little additional information about the genic effects for wean-

TABLE 3. REGRESSION COEFFICIENTS FOR THE INDEXES PREDICTING THE GENIC EFFECTS FOR TOTAL FEEDLOT GAIN (G_t') AND THE CORRELATIONS BETWEEN G_t' AND EACH INDEX

Index	P_1	P_2	P_3	P_4	P_5	P_6	P_7	σ_I'	$r_{G_t'I'}$
I_1'	1.15	....	....	....	....	....	....	....	.40
I_2'	....	.20	....	....	....	....	....	....	.46
I_3'	....	....	.56	....	....	....	....	....	.39
I_4'	....	....	....	.78	....	....	....	....	.49
I_5'	....	....	....	....	.51	....	....	....	.31
I_6'	....	....	....	....	....	.46	....	....	.29
I_7'	....	....	....	....	....	....	.15	....	.09
I_8'	.81	.17	....	....	....	....	....	14.1	.54
I_9'	....	....	.53	.73	.49	....	....	17.8	.68
I_{10}'	....	....	.53	.70	.48	.38	—.04	18.8	.72

ing weight when weaning weight itself is known.

When selecting for final weight it would be desirable to cull at weaning some calves with low weaning weights if this can be done without decreasing seriously the ultimate progress expected from selection. The optimum amount of early culling depends on the relative cost of the post-weaning evaluation and the difference in reach or selection differential if all culling had been done at the end of the feeding period. The latter is determined in part by the correlation between the index and weaning weight. For I_{19} this correlation was 0.64. Reach is defined as the average of the selected group minus the average of the calves before selection was practiced.

The reach on the index (I_{19}) resulting from culling first on weaning weight and later on the index was examined empirically using the 748 calves for which I_{19} could be computed. The reach in units of I_{19} is shown in table 4 for various fractions of the sample of calves, following culling at several different levels. The upper value in each cell is the actual combined reach accomplished by the early culling indicated at the top of the column plus sufficient later culling to make up the total culling indicated at the left of the row. Both the fraction kept at weaning (a) and the fraction finally kept (b) are based on the number of calves weaned. Thus if 0.4 of the calves are selected on weaning weight, and a final 0.1 which have the best indexes are kept, the percent selected as having the best indexes is 0.1/0.4 or 0.25. The reach actually accomplished on the 748 calves with this kind of selection was 69 index units, whereas the theoretical value expected was 73 units.

TABLE 4. ACTUAL AND THEORETICAL REACH FOR VARIOUS FRACTIONS SAVED AT WEANING AND AT END OF TEST PERIOD

Fraction finally kept (b)	Fraction kept at weaning (a)									
	1.0	.9	.8	.7	.6	.5	.4	.3	.2	.1
.1	70[a]	70	70	70	70	70	69	68	65	49
——	75[b]	74	74	74	74	74	73	71	67	48
.2	57	57	57	57	56	56	54	51	39	..
——	60	60	59	59	59	58	56	52	38	..
.3	48	48	48	47	46	45	41	32	..	..
——	50	49	49	49	48	46	42	32	..	..
.4	40	40	40	39	38	35	26	..	..	..
——	42	41	41	40	38	35	27	..	..	..
.5	34	33	32	31	29	22	..	..	..	..
——	34	34	33	32	29	22	..	..	..	..
.6	27	27	26	24	17	..	..	..	..	..
——	28	27	26	24	18	..	..	..	..	..
.7	21	20	18	13	..	..	..	..	..	..
——	21	21	19	14	..	..	..	..	..	..
.8	16	14	10	..	..	..	..	..	..	..
——	15	14	10	..	..	..	..	..	..	..
.9	9	5	..	..	..	..	..	..	..	..
——	8	5	..	..	..	..	..	..	..	..

[a] Upper value in each cell is the actual reach.
[b] Lower value in each cell is the theoretical reach.

The theoretical reach accomplished from the first selection is $\frac{Z}{a} r\sigma$, where Z is the ordinate of the normal curve at the truncation point, a is the fraction kept, r is the correlation between weaning weight and I_{19}, and σ is the standard deviation of I_{19}. Computation of the later selection is more complex because the later fraction kept on direct index selection is $\frac{b}{a}$ where b is also a fraction of those originally present at weaning. Thus Z′ is the ordinate for truncation selection including the fraction b/a. In addition, the earlier selection reduces the variance in the index (Cochran, 1951), the reduced value being $\sigma^2[1-r^2\frac{Z}{a}(\frac{Z}{a}-t)]$, where t is the truncation point in standard units. Hence the reach from the later selection

is the product of the reduced standard deviation and $\frac{Z'}{(b/a)}$. Then the combined reach is the sum of that accomplished from the early and later selection. In reality, the distribution of indexes after the early selection is skewed by the elimination of a disproportionate number of calves whose indexes would have been low, so that the above formula tends to give an overestimate. This effect is scarcely noticeable in the present example and would be serious only when the two criteria of selection are very closely correlated and selection on both criteria is quite intense.

Note that the actual and theoretical reaches correspond very closely, the only noticeable discrepancies occurring for the combination of very mild early selection and very intense later selection. The comparisons are particularly close where most of the selection is practiced at weaning time. The reduction in reach for any given final fraction kept that is caused by early selection can be examined by reading across the appropriate row in table 4. For example, for 0.3 finally kept, the theoretical reach is 50 units if no selection is practiced until the end of the 3-month feed test. But if 0.5 of the calves are selected on weaning weight and the final reduction to 0.3 accomplished at the end of the feed test, the combined theoretical reach is 46 units. This is a small price to pay if testing is expensive or if the saving in time, effort and facilities can be redirected toward some aspect of the breeding program which might otherwise have to be ignored. In every case there is considerable advantage to saving a larger fraction at weaning than will actually be needed. This is illustrated for the example above by noting that if the best 0.3 at weaning had been selected, with no room allowed for later culling, reach for final weight is only 32 units. Allowing some room for later culling permits breeders to eliminate those calves whose weaning weights will be good enough to pass the first hurdle but whose later records will be poor.

The theoretically best index to use in selecting for final weight, after some earlier selection at weaning is not the I_{19} used here. The very best index will depend upon the intensity of selection at weaning. In general, when selection is very intense at weaning, relatively little emphasis will be placed on weaning weight in an index for final selection. These problems were solved theoretically by Dickerson and Hazel (1943) but were ignored here because they are not an important aspect of comparing the actual and theoretical reaches under the conditions specified for table 4.

The reach in index units as computed in table 4 represents the expected genetic improvement in weight during the total period under study, i.e., approximately the first year. The regression of genotype for weight during this period on the indexes is unity because of the way the indexes were computed. It seems reasonable that 0.5 of the bulls with good weaning weights could be tested and that herd sires for seed stock herds could come from the best 0.1, with a realized reach of at least 70 lb. Because of the low intensity of selection that can be practiced on females, a reasonable program for them is to select the best 0.8 on weaning weight without attempting later testing. In this case the realized reach would be 9.6 lb. Considering the halving nature of inheritance and a 5-year generation interval, a reasonable rate of genetic improvement for weight at one year of age seems to be about $(70+10)/(2)(5)=8$ lb. per year. The replacement rates used here are in reality considerably larger than are needed to maintain seed stock herds of constant size; hence, ample room is left for selection on other criteria.

Conclusions

The high genic covariances found in this study indicate that, to a large degree, the same genes affect gain in weight of beef cattle during different parts of the growing period up to a year of age. This suggests that selection for weight at a year of age may be made earlier in the animal's lifetime with little loss of efficiency of selection. Such a practice could reduce the cost of evaluating animals and perhaps eliminate the excessive fattening of breeding stock.

The results of this study indicate that a short post-weaning evaluation of about 3 months is adequate for selection for weight at a year of age and that some culling may be done at weaning. It should be remembered that the calves in this study were all from the same farm and were handled similarly even in the different years. During the post-weaning evaluation the calves were self-fed on a high concentrate ration. Studies similar to this should be done on cattle raised under different systems of management to determine the general applicability of the results. If the advantages for the short feeding period indicated by these data apply to other cattle and con-

ditions, the results suggest that post-weaning evaluation periods may be shortened without serious loss of efficiency for selection for gaining ability.

It would be desirable to know if heifers could be evaluated on a high roughage ration for their ability to gain on full feed of a high concentrate ration. Since the selection differential is usually small for females in cattle, evaluating them by full-feeding on a high concentrate ration may not be justified. In view of the normal reproductive rates for cattle, the variability in weight for age, and the genetic relationships involved, genetic improvement at the rate of 8 lb. per year for twelve-month weight appears feasible.

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ABSTRACTS OF PAPERS PRESENTED AT THE MEETING OF THE NORTH ATLANTIC SECTION, A.S.A.P., AUGUST 23–24, 1960, AMHERST, MASSACHUSETTS

1. RATIO FACTORS FOR ADJUSTING MONTHLY TEST DAY DATA FOR AGE AND SEASON OF CALVING. L. D. VanVleck and C. R. Henderson, *Cornell University, Ithaca, New York.*

Averages from 129,882 D.H.I.A. test day records were classified according to age in months at calving (60 classes), season of calving (3 classes), and stage of lactation (10 classes). These averages were analyzed by an unweighted squares of means procedure. Ages, seasons, stages, and the season by stage interactions were significantly different at the 0.01% level of probability for both milk and fat records. Ratio factors for adjusting monthly records to a common age and season basis were computed by the gross factor method from the means. These factors were analyzed by a similar procedure as used for the means except ony 30 age groups were considered. Ratio factors were significantly different for ages, seasons, stage of lactation, and all interactions between these effects. The ratio factors were also different for milk and fat.

2. ESTIMATION AND APPLICATION OF VARIANCE COMPONENTS FROM ARTIFICIAL INSEMINATION FERTILITY DATA. H. O. Dunn, L. D. VanVleck, G. V. O'Bleness and C. R. Henderson, *New York Artificial Breeders' Cooperative, Inc. and Cornell University, Ithaca, New York.*

The 60- to 90-day nonreturns and returns to 115,480 first services performed by 217 technicians with semen from 35 Holstein bulls were subjected to variance component analysis using Eisenhart's Model II for taking expectations of sums of squares. Each bull was collected from 6 to 14 times at weekly intervals between November 1958 and March 1959. The estimated variance components and the corresponding percent of total variance are as follows: technicians $\sigma^2a=0.00084$ (0.45%), bulls $\sigma^2b=0.00053$ (0.28%), collections $\sigma^2a=0.00019$ (0.10%), $\sigma^2ab=0$, $\sigma^2ac=0.00006$ (0.03%), $\sigma^2bc=0.00029$ (0.16%), $\sigma^2abc=0.00340$ (1.81%), and random error $\sigma^2e=0.18223$ (97.17%). The mean nonreturn rate was 75%. The estimate of total variance, given by p (1—p) or 0.75 x 0.25, is 0.1875, which checked closely with the total of 0.187538 obtained in this analysis. The relative magnitude of these estimates is in general agreement with those reported previously from this station. These estimates can be employed to determine the optimum size and design of an experiment for detecting treatment differences of specific magnitude at chosen levels of probability. Since the random error σ^2e is so large in relation to the other classifications, it is essential to obtain a very large number of services per treatment to be sure (80–95% confidence) of detecting treatment differences as small as one or two percentage units at the 5% level of probability.

3. APPROXIMATE WEIGHTING FACTORS FOR SELECTION INDICES. T. Heidhues, L. B. VanVleck and C. R. Henderson, *Cornell University, Ithaca, New York.*

The use of the selection index method in the selection of herd replacements and bulls for artificial breeding studs has serious limitations because of the large amount of computations involved, unless an electronic computer is available. To overcome this difficulty tables of weighting factor were computed for a fairly large number of possible combinations of various sources of information on relatives. The values used for heritability and repeatability were 0.25 and 0.45 respectively. The estimated breeding values estimated by this method agree closely with those estimated using an exact method as developed by Henderson. The correlation between estimated breeding values according to these two methods was r=0.97 for a small sample of 35 individuals. The use of these tables in various situations is illustrated.

4. EFFECTS OF —196° C. AND —76° C. STORAGE ON RESISTANCE OF BULL SPERM TO THREE FREEZE-THAW TREATMENTS. A. K. Fowler, B. W. Pickett, D. G. Gosslee and W. A. Cowan, *University of Connecticut, Storrs.*

Three collections from each of ten Holstein bulls were frozen to —76° C. and divided appropriately for storage in liquid nitrogen (LN) and Dry ice-alcohol (DI). Following a two-hour adjustment period, 6 ampules from each storage temperature were utilized to determine the survival after one, two and three routine freeze-thaw cycles. A similar procedure was repeated after a six-month storage period. Fertility data (60–90 day % NR.) were collected on approximately 33 ampules per ejaculate of semen stored at —76° C. Statistical analysis of the immediate freeze-thaw treatments indicated a difference between bulls ($P<0.01$) in their response to repeated freeze-thaw cycles ($P<0.05$). Analysis of the immediate and six-month, freeze-thaw cycles showed a difference of $P<0.01$ between bulls, treatments and time. A significant interaction between treatments and time was also observed. All correlations between spermatozoon survival and fertility were found to be nonsignificant. (P=0.05).

5. AMINO ACID SUPPLEMENTS AND GOSSYPOL TOLERANCE IN THE RAT. C. A. Cabell and I. P. Earle, *U.S.D.A. Agricultural Research Service, Beltsville, Md.*

Both weanling and protein depleted rats fed a basal cottonseed meal—corn swine diet show reduced weight gains when a critical level of gossypol is added to the diet. Significant reductions in this effect of gossypol have been obtained by small supplements of crystalline amino acids; e.g., lysine in the case of growing rats, and lysine, isoleucine, valine and threonine in the case of depleted rats. In one trial addition of 0.04% gossypol to the basal diet reduced weight gains of weanling rats 29%. By supplementing this mixture with 0.3% dl lysine HCl the weight loss decreased to 13%. Further nonsignificant reduction was obtained with supplements of other essential amino acids. In repletion trials with protein depleted rats supplements of all four of the above amino acids have reduced the depression in weight gain to zero. The total intake of the different amino acids by both

classes of rats was estimated from food consumption data and from available tabular values of amino acid contents of the feed ingredients. These calculations identify the possible limiting amino acids when compared with known rat requirements. The experiments provide evidence that the relief of stress when these limiting amino acids are supplied is the reason for increased tolerance to gossypol. This is in contrast to the theory that a reaction of amino acids and gossypol occurs, but is in agreement with recent feeding experiments with swine which indicate that stress due to disease and/or environmental conditions reduce gossypol tolerance in these animals.

6. INFLUENCE OF GROWTH HORMONE (STH) AND LACTOGEN IN THE LACTATING RAT. G. J. Macdonald and R. P. Reece, *New Jersey Agricultural Experiment Station, New Brunswick.*

Virgin 200-gm. Hooded Norway rats were fed Purina Fox Chow fortified with 10% meat scraps. At parturition their litters were standardized to 8 pups per dam divided as equally as possible by sex. Subcutaneous injection of dams with STH (a gift from the Endocrinological Study Section, National Institute of Health) began on the fifth day of lactation in doses of 0.25, 0.50, and 1.0 mg./0.1 cc. daily. A control group was similarly injected with distilled water. Lactogen (Squibb, Luteotrophin) injections began on the second day of lactation. This group of dams received 2 mg./0.2 cc. daily. A sixth group received 0.50 mg./0.1 cc. of STH in addition to lactogen. Litters were weighed on days 1, 5, 6, 11, 14, 17, and 21. STH injected dams and controls were weighed on these days and their whole and eviscerated carcass weight obtained on day-21 from which the percentage of fill was calculated. Litter weight indexes, used to indicate lactations performance, were obtained by subtracting day-5 litter weights from day-17 litter weights. Dam weight changes were calculated similarly. All doses of STH caused significant dam-weight gains ($P<.01$). The 1.0 mg. dose response was significantly larger ($P<.01$) than either the 0.25 or 0.50 mg. response which were not statistically different from each other. The percentage of fill of STH groups was similar to that of the control group, indicating that body weight increase represented growth. Litter weight indexes of STH and water injected groups were not statistically different. Treatment comparisons of litter growth indexes showed lactogen vs. no lactogen treatments were significantly different ($P<0.05$). This indicates that lactogen was galactopoietic, whereas STH, either alone or in conjunction with lactogen, was not.

7. AGE AND PRODUCTION PARAMETERS OF RECORDS OF A. I. DAUGHTERS. L. H. Wadell, L. D. VanVleck and C. R. Henderson, *Cornell University, Ithaca, New York.*

A previous report on 54 sires being used in artificial insemination indicated that the variances and repeatability of these records, expressed as deviations from their adjusted stablemate averages, were non-homogeneous from sire group to sire group. This group of sires with 40 or more first lactation daughters had a total of 27,534 records in this analysis. If this non-homogeneity is something other than a sampling artifact it could be important in the prediction of the production of a sire's future daughters or in culling of cows based on their estimated real producing ability. Age correction factors were investigated as being a possible cause of this non-homogeneity. The average age at first calving of the daughters of a sire was found to vary significantly ($P<0.01$) from sire group to sire group. This could be a result of the interaction of the time a sire is brought into service with the desire of a farmer to have his cows calve in the fall. The variances and repeatability of the 305-2X-ME records were non-homogeneous ($P<0.01$) from sire group to sire group indicating that the age correction factors, if they were in extreme error, could be causing the non-homogeneity. However, the variance and repeatability of the actual records were also statistically different ($P<0.01$) indicating that this is not the case.

8. LABOR REQUIREMENTS FOR MAINTAINING DRY BEEF BROOD COWS ON AN OUT-WINTERING, SELF-FEEDING SYSTEM. W. A. Cowan, N. S. Hale and D. A. Grant, *University of Connecticut, Storrs.*

Accurate records have been maintained for parts or all of five wintering periods starting with 1955–56 on the labor requirements for dry beef cows on an out-wintering, self-feeding system. All labor necessary for checking cattle, spraying, treatment, snow removal, moving the feeding gate, silo cover and removing manure and spoilage was recorded. The ration consisted of free choice corn or grass silage from a bunker silo, water, salt and a mineral mixture. No hay or grain was fed, and no bedding was used. Labor requirements for recording periods of at least 6 weeks and up to the entire period yielded the following mean number of man-minutes of labor per cow per day for the five seasons 1955–56 through 1959–60, respectively: 1.38; 1.38; 1.01; 0.63; and 1.21. For a four-month period under such a system, calculated labor requirements would range from as little as 1.31 to as high as 2.87 man hours per cow.

9. NUTRITIVE EVALUATION OF VARIOUS SILAGES FED TO STEERS. Richard H. Cordts, Jr. and George W. Vander Noot, *New Jersey Agricultural Experiment Station, New Brunswick.*

A total collection digestion trial with four Hereford steers was conducted to evaluate four different silages: brome, orchardgrass, barley, and oat. A conventional 4 x 4 Latin square designed was employed with 10-day preliminary and 7-day collection periods. Although the gross nutrient content of the silages did not vary greatly, the T.D.N. content of each of the grain silages was superior to the other two. Statistical analysis revealed the only significant difference ($P<0.05$) in the D.E. of the silages was the superiority of the barley over the brome. The greater consumption of the barley silage, indicating a higher degree of acceptability, resulted in the steers receiving approximately one-third more energy. The correlation of 0.99, shown to exist between the T.D.N. and D. E. of the four silages, was highly significant ($P<0.005$) for 14 degrees of freedom. A regression equation of $Y=0.043+0.0424X$, where Y is Cal./gm. of dry matter and X is T.D.N. percentage, was developed from the data obtained from this trial. A value of 4.31 Cal./gm. of T.D.N. (dry basis) was calculated from this data. This figure is slightly lower than what has been found by other workers, and only

further investigation will reveal if this is characteristic of silages.

10. THE RELATIONSHIP OF TOTAL DIGESTIBLE NUTRIENTS AND DIGESTIBLE ENERGY VALUES OF SILAGES CUT AT TWO STAGES OF MATURITY. Richard T. Hunt and George W. Vander Noot, *New Jersey Agricultural Experiment Station, New Brunswick.*

A digestion trial with four Shropshire wethers was conducted for the purpose of further elucidating the relationship between T.D.N. and D. E. In addition, the trial was designed so as to permit the comparison of the nutritive values of silages produced from forages cut at two stages of maturity. The silages and their stages of maturity were: (1) corn —early dent and late dent; and, (2) alfalfa—one quarter bloom and full head. Analysis of the resulting data yielded a correlation coefficient of +0.99 between T.D.N. and D. E. This was highly significant ($P<0.01$) for 14 degrees of freedom. The regression equation $Y=114.99+40.11X$ was calculated where Y is the D.E. value expressed in calories per gram of feed and X is the T.D.N. percentage. The increasing maturity of the forages was accompanied by various changes in the chemical composition of their dry matter. While resulting in an increase in T.D.N. and D.E. and a decrease in digestible protein in corn, and a decrease in T.D.N., D.E. and digestible protein in alfalfa, the changes were of insufficient magnitude to cause any significant differences ($P<0.05$) in nutritive value between the two stages of maturity of either silage.

11. EFFECTS OF VARIOUS FATS ON FEEDLOT BLOAT. C. J. Elam and R. E. Davis, *U.S.D.A., A.R.S., Beltsville, Md.*

Eight steers with known bloat history were randomly assigned to two squares of 4 x 4 Latin square experiment. A bloat-producing ration of the following composition served as the basal ration: barley, 61%; soybean meal, 16%; dehydrated alfalfa, 22%; and NaCl, 1%. All steers had bloated previously on this ration. The four treatments were: (1) basal ration (B); (2) B with 8% soybean oil (SO) replacing an equal weight of barley; (3) B with 8% mineral oil (MO); and (4) B with 8% animal fat (AF). The rations were pelleted and were fed twice-a-day during each of the four 42-day periods. Four time a day steers were given bloat ratings of from 1 (no bloat) to 4 (severe bloat). Average bloat ratings for the eight steers were 2.06, 2.40, 1.49, and 2.07 for treatments B, SO, MO, and AF, respectively. Adding SO to the basal increased ($P<.01$) bloat while the addition of MO decreased ($P<.01$) bloat. Animal fat did not affect the incidence of bloat. Ruminal pH and ingesta volume increase (IVI) were not altered by treatments, but stable IVI was higher ($P<.01$) when steers were fed AF and lower ($P<.05$) when fed MO than for either B or SO. Neither the total concentration of volatile fatty acids (VFA) in ruminal samples nor the proportions of the acids were altered by treatments. The average concentration of total VFA was 150.1 m mol./1. with the following proportions: acetic acid, 58.0; propionic acid, 23.6; and butyric plus higher acids, 18.4. Blood fat was higher ($P<.01$) when steers were fed SO or AF than when they were fed B or MO.

12. ENZYME ADDITIONS TO LOW-MOISTURE CORN FOR FINISHING STEERS. Jesse F. Smith and A. F. Kish, *Pennsylvania Farm Bureau Cooperative Association, Harrisburg.*

Thirty-nine Good and Choice 800-lb. Angus steers were divided into four groups (9 in control). Weight gains, grade and grain feed efficiency were measured. The basal ratior was a low-moisture corn (—14%), low quality grass hay, and a 50% commercial supplement containing diethylstilbestrol. To each treatment was added one of three different enzyme combinations in the supplement at the .0075% level. Proteolytic and amylolytic enzymes were the primary enzymes in each combination. (Zymo-Pabst, Pabst Brewing Co., Milwaukee, Wis; Agrozyme, Merck & Co., Rahway, N. J.; Dawe-Nzyme, Dawe's Laboratories, Inc., Chicago, Ill.) The average weight at the end of the 126 day trial was 1,090 lb. The treated groups had a weight gain improvement ranging from 4.3% to 11.3% and a grain feed efficiency improvement ranging from 2.9% to 9.8% over the controls. There were no significant differences in live or carcass grades between control and treated groups. The dressing percentage for controls and treated groups was 61.55%.

13. EFFICIENCY OF GAINS OF BEEF CALVES ON CORN SILAGE AND SOYBEAN OIL MEAL. Nathan S. Hale, W. A. Cowan, and D. A. Grant, *University of Connecticut, Storrs.*

Twenty-eight bull, heifer and steer calves of the Hereford and Angus breeds were weaned on November 13, 1959 and started on a ration, fed in bunks once daily, of 27% moisture corn silage available free choice, 2.5 lb. of 41% soybean oil meal and a mineral mixture and water also available. During the adjustment period, all calves were treated for internal and external parasites and steer calves (12) implanted with 36 mg. of diethylstilbestrol. The trial started November 30, 1959 and ended April 4, 1960, a period of 126 days. At the start of the trial, Groups I, II, and IV had oleandomycin added to their soybean oil meal at a level of 20 mg. per head per day. Group I contained seven bulls averaging 424 lb. at start; 699 lb. at end; 2.19 lb. daily gain; 31.6 lb. daily silage consumption; and feed cost of $0.115. Group II contained six steers averaging 487 lb. at start; 765 lb. at end; 2.21 lb. daily gain; 36.92 lb. daily silage consumption; and feed cost of $0.126. Group III contained six steers averaging 479 lb. at start; 765 lb. at end; 2.28 lb. daily gain; 37.99 lb. daily silage consumption; and feed cost of $0.124. Group IV contained nine heifers averaging 391 lb. at start; 614 lb. at end; 1.78 lb. daily gain; 29.23 lb. daily silage consumption; and feed cost of $0.135. Corn silage was charged at $10.00 per ton and soybean oil meal at $75.00 per ton.

14. THE MARYLAND R. O. P. BULL TESTING PROGRAM. Boyd T. Whittle, *University of Maryland, College Park.*

Fifty-nine bulls from 22 Maryland herds were fed at a central location from November 19, 1959, to April 7, 1960. A ten-day "Conditioning" period was allowed prior to the official start of the 140-day test. Fifty-five of the bulls were sold at public auction one week after the test period. Requirements for the test stated that bulls were to have been born between September 1, 1958, and March 15, 1959. It was

recommended that bulls have a minimum growth record of 1.8 lb. per day at the time they were placed on test. Similar requirements for performance on test were adpoted, but some leniency on the part of the sale committee was exercised. This project was sponsored cooperatively by the Maryland Beef Cattle Improvement Association, the Animal Husbandry Department of the University of Maryland, the Maryland Livestock Sanitary Service and the Howard County Fair Association. Two barns typical of those used to house cattle at fairs were divided into four pens, and bulls were allocated according to weight. A mixed ration containing corn and cob meal, molasses, ground alfalfa hay, and protein supplement was fed free-choice. Individual weights were taken at the beginning of the test and at 28-day intervals thereafter. All bulls were given a type score at the beginning of the test and at the end. Average daily gains on test ranged from 1.57 lb. to 2.75 lb.

15. EFFECT OF NITROGEN FERTILIZATION AND DATE OF HARVEST UPON THE NITROGENOUS COMPONENTS OF ORCHARDGRASS. B. R. Stillings, J. W. Bratzler, E. Keck, Jr. and L. F. Marriott, *Pennsylvania Agricultural Experiment Station, University Park.*

First-cutting orchardgrass (S-37), replicated four times for each level of N fertilization, was harvested in 1958 and 1959 at three stages of maturity in which there was an eight to ten-day interval between the harvest dates for each treatment. Levels of N fertilization used in both years were: 50, 100, 200-split, 200 and, in 1958 only, 300 lb. per acre applied as ammonium nitrate. The total N in the forage samples, on the dry matter basis, ranged from 1.57—4.66% in 1958 and 1.84—4.96% in 1959. The nitrogenous components were determined and the following ranges, expressed as percent of the total N, were found in 1958 and 1959, respectively: non-protein N 20.5—34.8, 23.1—36.6; nitrate N 0.0—15.9, 0.0—11.5; amino N 9.3—13.7, 8.9—15.6; ammonia N 1.4—2.2, 0.7—1.2; hydrolyzed N 1.0—1.8; and other N 7.1—14.9, 7.0—15.0. The hydrolyzed N was determined only in the 1959 forages and was considered to be primarily amide N. The decrease in ammonia N found in the 1959 forages was attributed to losses during drying. The total and nitrate N increased, and the non-protein and ammonia N tended to increase with increasing applications of N fertilizer. On successive dates of harvest, the total N decreased, the nitrate N tended to decrease and the non-protein N tended to increase. The forages grown with 200 and 300 lb. of N contained KNO_3 equivalent values as high as 5.3% of the dry matter and were above 1.0% in all cases.

16. RUMEN DIGESTION OF FORAGES. J. G. Archibald, H. Fenner and H. D. Barnes, *Massachusetts Agricultural Experiment Station, Amherst.*

Comparative digestibility of alfalfa and timothy hay was determined *in vivo* with four rumen-fistulated mature Holstein cows by means of the so-called Dacron bag technique, in a double reversal trial extending over 24 weeks and involving four six-week periods. During the last three weeks of each period four Dacron bags, each containing 30 gm. of chopped hay, were placed in each cow's rumen for 48 hours, an arrangement being used which kept the bags suspended from the fistula cannula and weighted them so they would not float in the dorsal region of the rumen. Sufficient replications of the procedure were run so that results are available from 96 individual trials for each kind of hay. Results obtained for dry matter digestibility only form the basis of this paper. These were remarkably uniform; the average digestion coefficient for alfalfa dry matter was $64.4 \pm .4$ and for timothy $65.4 \pm .6$. The difference was not significant ($P > .1$). Sixteen conventional digestion trials by the total feces collection procedure were run on the same lots of hay with the same cows immediately after the periods of bag placement. The average dry matter digestibility of the alfalfa hay was $58.4 \pm .4$, of the timothy 63.4 ± 2.2. This difference was highly significant ($P < .005$). Personal observation leads to the belief that this lack of agreement between the two methods is due to greater leaf loss in the alfalfa as fed during the conventional trials than in the core samples obtained from intact bales for use in the Dacron bags.

17. VOLATILE FATTY ACIDS IN THE RUMEN LIQUOR OF BULLS ON RECORD OF PERFORMANCE TESTS. P. A. Putnam, K. Bovard and R. Priode. *U.S.D.A., A.R.S., Beltsville, Md., and Virginia Agricultural Experiment Station, V.P.I., and Beef Cattle Research Station, Front Royal, Va.*

Rumen liquor for volatile fatty acid analysis was obtained from bulls on ROP test at the Front Royal Beef Cattle Experiment Station. A stomach tube was used to obtain the samples. Twenty bulls were sampled in 1959 and 23 were sampled in 1960. Each animal was sampled twice (28 days apart) near the end of the 168-day ROP test, and the values for the two samples were averaged. In 1959, the animals had feed available prior to sampling but did not have access to water. The reverse was true in 1960. Butyric plus higher, propionic, acetic and total acids were determined chromatographically. Correlation coefficients were calculated for average daily gains and concentrations of the various volatile fatty acids. The data for 1959 indicated a significant negative correlation ($r = -.469$) between average daily gain and the concentration of acetic acid. All the other r values were negative but none was significant. The data for 1960 revealed no significant correlations between average daily gains and concentrations of volatile fatty acids. When the 1959 and 1960 data were pooled the r-value for average daily gain and acetic acid was not significant. The variation among samples was greater in 1960. This suggests that the availability of feed prior to sampling may be less critical than the availability of water.

18.

19. EFFECT ON SHEEP PROLIFICACY OF POST ESTRUS TREATMENT ON DAY 12, 14, OR 15 WITH PREGNANT MARE SERUM. D. A. Grant, W. A. Cowan and N. S. Hale, *University of Connecticut, Storrs.*

Over a two-year period, 30 Dorset ewes were employed in a reversal trial to determine the effect of 400 r.u. of P.M.S. injected on the 14th day following previously recorded estrus on lambing percentage. Vasectomized rams were used to determine the estrus cycles on all ewes. In 1958 the average percent pro-

lificacy was 129 and 208, respectively. In 1959 it was 146 and 191, respectively. In a second trial, 26 Shropshire ewes were divided into three groups to check the range, in days, which P.M.S. could be used to increase the prolificacy of the ewes. The three groups were (1) Control, no treatment; (2) Experimental I, injected with 400 r.u. of P.M.S. on the 15th day following recorded estrus; (3) Experimental II, injected with 400 r.u. of P.M.S. on the 12th day following recorded estrus. The average percent prolificacy was 160, 150 and 240 for control, Experimental I, and Experimental II, respectively.

20. EFFECT OF THREE MANAGEMENT SYSTEMS ON THE GROWTH OF LAMBS AND DEVELOPMENT OF INTERNAL PARASITISM. I. L. Lindahl, J. H. Turner, K. C. Kates, G. E. Whitmore and F. D. Enzie, *Agricultural Research Service, U.S.D.A., Beltsville, Maryland.*

From March to September of 1959, three bands of lambs were raised on dry lots, "clean" pastures, and parasite-contaminated pastures, respectively, and were kept under parasite surveillance. Each band was thus exposed to low, moderate, and high levels of parasitism, respectively. All lambs were on phenothiazine-salt prophylaxis. The principal parasites observed in the pastured lambs were *Haemonchus contortus* and *Strongyloides papillosus*, which caused anemia and inhibited growth. In the dry-lot group, significant parasitism did not develop, and therapeutic medication was unnecessary. The lambs exposed to high levels of parasitism required additional medication in June, whereas those exposed to moderate parasitism did not require therapeutic medication until late summer.

21, 22, 23, 24

25. SILAGES IN RATIONS FOR BROOD SOWS. James L. Gobble, *Pennsylvania Agricultural Experiment Station, University Park*

In a feeding trial with yearling sows during the winter gestation period, eight animals were fed 6 lb. per head per day of a complete gestation ration from 3 weeks after breeding to the 110th day of gestation at which time they were placed in farrowing pens. Seven similar bred sows were handled in a like manner except that each received daily about 7.5 lb. soybean silage plus 1.5 lb. ground shelled corn and 1 lb. of a highly fortified protein supplement. The silage-fed sows farrowed 11.0 pigs and weaned 9.9 pigs per litter with an average litter weight at weaning of 329 lb. The control sows farrowed 10.3 pigs and weaned 7.7 pigs per litter with an average litter weaning weight of 304 lb. Feed costs per pig at farrowing were $1.25 and $1.96 for pigs from the silage-fed sows and control sows, respectively. In another trial, 18 sows divided equally into two lots were fed 6 lb. of control ration and 10 lb. of corn silage plus 3 lb. of a mixture of equal parts of ground shelled corn and protein supplement, respectively. The results from the silage-fed and control-fed sows, respectively, for the litter size at farrowing and weaning, litter weight at weaning, and average feed cost per pig at farrowing and weaning are: 11.3, 11.1; 9.9, 9.7; 425, 424; $1.43, $1.66; $6.34, $6.57. These data indicate that when soybean or corn silages are available on the farm for feeding other livestock, they may be fed successfully to brood sows during winter gestation in place of pasture with a saving of considerable grain.

26. COORDINATED APPROACH TO PROFITABLE SWINE PRODUCTION IN PENNSYLVANIA. Lester A. Burdette, *Pennsylvania State University, University Park.*

Efforts to increase swine production and carcass quality in Pennsylvania are being guided by a coordinated approach. Representatives of extension, teaching, and research from various subject matter sections are involved. Attempts are being made to involve county agents, vocational agriculture teachers, feed fieldmen, meat packers, and veterinarians in this program. A Swine Handbook has been written by members of the committee in an effort to standardize recommendations by the various information mediums throughout the state.

27, 28

29. PHOTOGRAPHS FOR CARCASS EVALUATION. J. R. Ferguson, M. C. Brennan and J. Buric, *University of Maryland, College Park.*

A 4 x 5 Press Camera equipped with a Polaroid attachment that accepts 4 x 5 in. Polaroid Land Film packets, a stainless steel grid, and a 100-Watt bulb for light, was used to photograph trimmed pork loins. Area of loin eye muscle was determined from the photo. Pictures were taken of the exposed rib eye of ribbed beef carcasses hanging on the rail. Thickness of fat covering and area of lean meat were determined. External fat and rib eye area of lamb carcasses were also obtained. Photographs are more accurate than tracings since they eliminate human error in tracing, correct for angle of cutting, and provide a permanent record of the lean-fat relationship.

30. VARIATIONS IN TENDERNESS OF A NUMBER OF MUSCLES WITHIN THE BEEF CARCASS. Donald M. Kinsman, *University of Connecticut, Storrs.*

Sixteen different muscles of the round, loin, rib and chuck from U. S. Standard grade beef carcasses were evaluated for cooking time, cooking loss and tenderness in a preliminary study conducted at the Oklahoma State University meat laboratory. Two-inch steaks were cut from each of the following muscles: *semitendinosus, semimembranosus, biceps femoris, vastus lateralis, adductor, rectus femoris, ilio psoas, gluteus medius, psoas major, longissimus dorsi, triceps brachii, infraspinatus, spinalis dorsi, serratus ventralis, rhomboideus* and *supraspinatus*. Each steak was frozen at —20° F. and stored at 0° F. for about 2 months and thawed for 24 hours at 40° F. before cooking in deep fat at 275° F. to an internal temperature of 150° F. Three, one-inch cores were sheared in triplicate from each steak by the Warner-Bratzler shear machine. Cooking time and percent cooking loss were also determined. Shear values ranged from 10.8 (*L.D*) to 21.8 (*rhomboideus*) and percent cooking loss from 20.2% (*L.D.*) to 39.4% (*serratus ventralis*). This pilot study was not of sufficient scope to apply statistical analysis but did indicate a difference in cooking time and percent cooking loss between muscles as well as tenderness differences as expressed by shear values. Variations in tenderness within muscles was also noted.

31. CASEIN AND ITS DEGRADATION PRODUCTS IN THE UTILIZATION OF UREA NITROGEN BY LAMBS. G. A. McLaren, G. C. Anderson, K. M. Barth and J. A. Welch, *West Virginia University, Morgantown.*

Two metabolism trials were conducted with wether lambs (32 kg.) to determine the influence of additions of intact (I), acid hydrolyzed (A) and enzyme hydrolyzed (E) casein upon digestibility and nitrogen utilization. The isonitrogenous, semipurified rations fed were composed of a purified concentrate mixture, blackstrap molasses and corn cobs, and contained 1.7% nitrogen, 87% of which was supplied by urea and/or the casein material. Each trial consisted of a 10-day preliminary and a 10-day collection period. In trial I 10% of the supplemental nitrogen was supplied by I, A and E in the various combinations of each permitted by 1, 1, 8, proportions. None of the treatments resulted in a significant response although the combination containing 8% E improved nitrogen utilization. In trial II 4, 8, and 16% of the supplemental nitrogen was supplied by E. None of the treatments resulted in a significant effect. A combined evaluation of both trials revealed that 8% of E significantly improved dry matter digestibility and nitrogen utilization.

32. EFFECTS OF PHOSPHORUS, SULFUR, AND MOLYBDENUM SUPPLEMENTATION OF A STEER RATION ON CELLULOSE DIGESTION AND ON THE RUMEN LIQUID CONCENTRATIONS OF PHOSPHORUS AND SULFUR. Joe L. Evans and George K. Davis, *University of Florida, Gainsville.*

Experiments were carried out to determine the effects of various concentrations and combinations of dietary phosphorus, sulfur, and molybdenum and of steer variation on the rumen liquid concentrations of phosphorus and sulfur and on cellulose digestion. Steer variation appeared to have less effect than dietary mineral concentrations on rumen liquid concentrations. With dietary phosphorus concentrations of 0.04, 0.16, and 0.54%, rumen liquid concentrations were 0.83, 2.28, and 2.97% on a dry matter basis and 187, 410, and 561 mcg. per ml., respectively. Also, dietary sulfur supplementation increased the rumen liquid concentration of sulfur. The 0.54% dietary phosphorus did not affect intake, but it effected a reduction in cellulose digestion. Although added sulfur increased cellulose digestion, ingestion was slower. Molybdenum supplementation of the basal ration in the absence of added phosphorus or sulfur initiated a reduction in ration intake.

PROCEEDINGS OF THE 52nd ANNUAL NATIONAL MEETING OF THE AMERICAN SOCIETY OF ANIMAL PRODUCTION

The meeting was held in the Hotel Sherman, Chicago, on November 25–26, 1960, the general session convening at 11:15 a.m. the first day and divided sessions for presentation and discussion of research papers in Breeding and Genetics, Extension, Teaching, Meats, Nutrition, Pasture and Forage, and Physiology being held from 9 to 11 a.m. and from 1 to 4 p.m. the first day, and from 9 a.m. to 4 p.m. and from 7:30 p.m. to 10 p.m. the second day.

The annual dinner was held at 6 p.m. November 25, with H. H. Stonaker, Vice-President, in charge. The annual business meeting with J. K. Loosli, President, presiding was held immediately after dinner.

GENERAL SESSION

Introductory Remarks

J. K. Loosli, *President*

Members of the Society and Guests:

It is a pleasure to welcome you to this 52nd Annual Meeting of the American Society of Animal Production.

I should like to express the sincere thanks of the officers of the Society to all members who have served on the various committees during the year and to those who have done much to organize this meeting and carry it forward. A special word of appreciation is due the Secretary-Treasurer and Business Manager of the Journal of Animal Science, Dr. C. E. Terrill, for the tremendous amount of effort he has devoted to Society business. The Editor and Chairman of the Editorial Board, Dr. W. D. Gallup has given generously of his time and talents the past three years and we express our sincere thanks to him. Without such devoted service the Society and the Journal could not have grown to their present status. The Associate Editor, Dr. A. B. Chapman, has assumed the responsibility for editing the special Monograph on "Techniques and Procedures in Animal Production Research". Our congratulations go to him and the authors of the various chapters on the publication after 10 years effort. I am sure it will prove useful for even longer than a decade.

It is a pleasure to advise you that your Society continues to grow, having added 219 new members since last year, bringing the present membership to 2169. Interest of scientists from countries other than the U.S. and Canada continue to increase.

It has been an honor to serve as your President during 1960 and I extend my thanks to all members of the Society for this privilege. One of the rewards of this position is the opportunity provided to work with members of the Society and with officers of other scientific organizations throughout the world.

Your president had the honor during August and September to travel to Australia on a Fulbright grant at the invitation of Dr. T. K. Ewer, President of the Australian Society of Animal Production to represent your Society and be guest speaker at the biennial meeting of the Australian Society. I bring you greetings from your sister Society in Australia.

More American animal scientists are traveling abroad each year. Many are giving assistance to other countries on various governmental and Foundation programs. Modification of our training schedules would make our younger scientists more aware of world problems and better qualified to share our knowledge with less developed countries. The program this morning is planned to stimulate awareness of opportunities for foreign service in helping America grow.

We are fortunate, indeed, that our guest speaker today is Dr. Harrar, vice president of the Rockefeller Foundation. A native of Ohio, he received his training at Oberlin

College, Iowa State University and the University of Minnesota, with emphasis during the Ph.D. program on plant pathology. He has taught and conducted research at Puerto Rico, Minnesota, Virginia Polytechnic Institute (VPI) and the State College of Washington before joining the Foundation. He has been in charge of the Foundation's Agricultural program in Mexico and more recently in South America as well. At present he is in charge of the entire agricultural program. He has been given medals of merit for his contributions to agriculture by the governments of Mexico and Columbia. It is an unusual pleasure to present Dr. J. G. Harrar,[1] vice president of the Rockefeller Foundation.

[1] Dr. Harrar's address "American Science Overseas" to the Society is not published.

PRESIDENT'S ADDRESS

REDIRECTING EDUCATION IN ANIMAL SCIENCE

J. K. Loosli, *Cornell University, Ithaca, N. Y.*

November 25, 1960

In the 52 years since it was organized this Society has afforded leadership to the animal industry. It has provided an annual forum where extension teachers of adults, classroom teachers of college students, research men and administrators could air their views and discuss their mutual problems. Through its publications it has kept the world informed of the scientific progress and advances in all phases of livestock production in the U.S. and Canada.

On December 1, 1942, R. M. Bethke, then president of the American Society of Animal Production, began his address (2) at the annual meeting as follows! "This, the thirty-fifth annual meeting of our Society, finds our country in alliance with other nations of the world actively engaged in a war to preserve the principles of a free democracy". If we would insert the word "cold" before war that statement would apply as well today, 18 years later. With our allies we won that war, but many have told us we lost the peace—and that we are losing the cold war. Various people have been blamed. The press has freely criticized government agencies, schools and other organizations for our supposed plight.

Especially since "Sputnik" we have heard a lot of criticism of the American educational system. An example of the dissatisfaction with higher education is found in the report of a special committee that studied the University of the State of New York during the past 10 months. The report recommends, in addition to expansion to handle twice as many college students by 1970, changing most of the teachers colleges in the state system to liberal arts colleges so that future teachers being trained for our elementary and high schools can be taught more basic information rather than spending so much time learning methods of teaching subjects they don't know.

Jacques Barzun, Provost and Dean, Columbia University, in an address before the Graduate School at Cornell University (1) indicated that "our elementary and high schools and to some extent our colleges, have been directed toward every good purpose ahead of an intellectual purpose. It has been for character, citizenship, health, social and individual adjustment, cultural assimilation, vocational aid, profitable friendships, marriage opportunities and hobbies of distinction". Some say our colleges serve mostly to correct the deficiencies of high school graduates.

President Malott of Cornell University aptly states (3), "To the extent that our secondary schools short-cut the basic fundamentals essential for preparation for college and university, we have cheated ourselves of some of the leadership of tomorrow. Our young people who have been traded out of the fundamentals of mathematics and science, of language and literature, for the bogus lures of courses in life adjustment—whatever that means and is—may never realize the potential that might have been theirs. For if any one thing is palpably clear about the

present state of our society, it is that greater and more intensive mental effort will be necessitated by us all."

Some of the criticism is certainly justified and measures already have been instituted to improve and accelerate educational programs.

The American Ph.D. has come in for its share of criticism, and we recognize that it must be strengthened constantly. This degree in the animal sciences, however, is becoming more widely distributed and is more highly prized than ever before.

I wish you could all know how much an American education is desired and sought after by thousands of young people in India, the Middle East, Philippines, and other far eastern countries, South America and other free countries of the world. Even in Europe and the United Kingdom today, an American advanced degree greatly improves the status and usefulness of the person who is fortunate enough to be able to earn one. I wish you could all see the numerous letters from students of other countries pleading for minimum financial assistance so they can come to America and study, and for many of them we can offer nothing. What an opportunity we are missing to serve the less developed countries and spread the ideals of freedom.

Even in my limited experience I have traveled enough to know that a vision of a dynamic and progressive America still exists in many of the countries of the world in spite of efforts to erase it. This is true because some people have heard of our abundant, cheap and high quality food in contrast to their hunger, our comfortable homes and clothing and our material wealth, but even more I believe, is the vision of health and medical care and of education for everyone with the opportunity it offers to improve one's position.

We know, of course, that our abundance of food and material things carries the stigma of surpluses, and right now this looms up like a double headed monster, greatly disturbing agriculture and especially animal science. In view of these surpluses and the cost of the subsidy problem it has been proposed by the uninformed that agricultural research and extension programs should stop until consumption overtakes production.

An opposite view has also been expressed, that the population is increasing so fast that before long we will have trouble producing enough food. On this point Max Kleiber believes that, given adequate opportunity, the earth can be made to produce all the food needed by future generations. Our main problem will be one of providing living space, or elbow room. I like that thought. The question, therefore, becomes one of how many people can learn to live together in the area available, and not how can we ever feed so many people? But can we learn to live together? What about our racial problems? Can we give other countries the impression we want only to help them help themselves?

Leadership Is an Important Need

The assistance and leadership provided by the Food and Agriculture Organization illustrates the potential for improving the food production of less developed countries (6).

One thing we in America must all recognize is that the free world, and especially the less developed countries want us to assist them by providing creative leadership. We have a tremendous opportunity to serve the cause of world peace by exporting some of our technology. Modern "know how" can rapidly be applied to food production to solve one of the big problems of many of the African countries. Upgrading the diet to correct and prevent nutritional deficiencies still common in these countries, presents a great challenge to scientists in animal production and in all agricultural technology. The latent potentials in these areas can quickly be realized with some friendly cooperation.

There are two ways of exporting our technology. One is to send our scientists and technologists abroad to help train the local workers and to organize training, research and production programs. The other way is to accept advanced students from other countries for training in our universities. Both of these are being done now, but we could greatly expand this type of service to other countries.

Under the British, French and Dutch, higher education was reserved for a very few. As a result many countries today in Asia and Africa have an appalling shortage of educated leaders. This largely explains today's problem in the Congo where Belgium failed to educate the natives for self government. Russia and China are not only willing but anxious to fill the gap. Where do we stand?

The main purpose of an animal industry is to provide human food, although of course, wool, and skins and byproducts also have some importance. The breeding, selecting,

feeding and managing of livestock should all be aimed at increasing the quality of the products and the rate and efficiency of producing these products.

The healthiest and most progressive peoples of the world in the past have been those who could have a certain amount of animal products in their diets. Protein and vitamin deficiencies are largely prevented when adequate amounts of animal products are consumed.

Wide differences are found among various countries in the use of animal products. Let me cite one example. Dr. V. A. Oyenuga, Professor of Animal Nutrition, University College, Ibaden, Nigeria (4) has estimated that the amount of meat available in Nigeria from beef, goats, mutton and pork is 15.6 lb. per capita per year. Contrast this with the 159.6 lb. per capita consumed in 1959 in the U.S. plus an additional 35.8 lb. from poultry and turkeys. In Nigeria an average of 16 lb. of milk were available yearly for each person and 5.6 lb. of fish. Poultry and eggs are also scarce and expensive.

Considering these wide differences in amounts of animal products in the diet it is very interesting to note that in North America there are only about twice as many cattle per capita as in Nigeria and the numbers of sheep are no longer on this basis. Clearly, therefore, the main difference in the availability of food products from these sources lies in the productivity of the animals—e.g. the efficiency of reproduction, the growth rates and the rates of turnover and processing.

This wide difference could be removed as the result of research to improve the type of animals and their performance. Education of young men to do the research and of those who care for the animals is essential before the desired productive efficiency can be achieved. We have developed in America one of the most efficient livestock industries in the world and others want to profit from our experiences.

Clearly we need to offer no apology for past performances in most areas of animal science, but past performance is not good enough in the years ahead. Most schools now recognize this and some are starting to revise course offerings to eliminate the folklore. These revisions will stress basics and not waste time on non-essentials that have to be relearned on the job after graduation. There will be greater specialization. As we make our training and research approaches more basic and specialized—as we must—let's keep in mind that we need more than biochemists, physiologists, geneticists and statisticians; we still need animal husbandmen who have an interest in applying latest scientific findings to animal production. Only in this way can we continue to attract graduate students of high potential. But we need to do more than this.

Last week President Eisenhower released a report of his Science Advisory Committee, headed by Dr. Glenn T. Seaborg, chancellor of the University of California. Among other things the report recommended the federal government should assign funds to the universities to "promote the essential connection between the conduct of research and the training of scientists" (5). In scientific education the two are "essentially inseparable". The report also said that in all but a few universities the teaching load of the professor is such as to make it difficult for him to carry on any serious program of investigation of his own. Many research scientists do not contribute to graduate teaching. A proper balance between the two is the only way that American science can maintain its position.

Money is not the only shortage. We must begin to do our share in educating the general public to the contributions of America's educational system and of the role it must fulfill in the immediate future if we are to contribute to world progress—or for that matter even survive. We must rekindle the concept that it is a privilege to defend America. We must return to the kind of personal honesty that makes us deliver a full day's work for a day's pay. We must recapture some of the frontier spirit of brotherly cooperation and mutual trust that will allow us to be our brothers' keeper in times of need, to speak up in defense of truth and right, and to make any personal sacrifice necessary to spread the cause of freedom and justice for all.

Let's make sure our American students are aware of their American heritage and know the merits of the measure of freedom we have, as well as what we must do to preserve it and give it to others.

Let's accept students from other countries. Train them in their scientific fields, but also show them how our free educational system works in the elementary and high schools; how much better a free election works than a military coup, how the American free press is superior to a controlled one, and how indi-

vidual freedom has made it possible for America to become great.

The Journal of Animal Science is read throughout the world. Let's recognize that the world is our campus.

References

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2. Bethke, R. M. President's address. J. Animal Sci. 2:80, 1943.
3. Malott, Deane W. Report of the President, Cornell University, Ithaca, N. Y., June 13, 1960.
4. Oyenuga, V. A. Our needs and resources in food and agriculture. Lugard Lectures, Fed. Min. of Information, Lagos, Nigeria, 1959. Also personal communication.
5. New York Times, Nov. 20, 1960, page 1. Report Bids U. S. Spur Science Aid for Universities.
6. Phillips, R. W. Food and agriculture organization completes 15 years. Science 132:871, 1960.

A SUMMARY OF THE MINUTES OF THE 52nd BUSINESS MEETING OF THE AMERICAN SOCIETY OF ANIMAL PRODUCTION

C. E. TERRILL, *Secretary*
Beltsville, Maryland

The 1960 Annual Business Meeting of the American Society of Animal Production was held in the George Bernard Shaw Room of the Hotel Sherman, Chicago, Illinois, November 25, 1960 at 8:20 p.m.

President J. K. Loosli called the meeting to order and requested approval of the minutes of the 1959 Business Meeting as printed in the Journal of Animal Science, Vol. 19, No. 1, 323–335, inclusive. It was moved by W. A. Craft that the report be accepted, S. D. Musgrave seconded, carried.

The Secretary's report was read by C. E. Terrill as follows:

"Continued and increased growth in membership, subscriptions, and in number of papers presented at the annual meetings has taken place in 1960. Membership in the Society increased to 2,169 representing a net increase of 219 in total membership. There are 2,029 members in the United States and Canada, and 140 in other countries. The latter include 36 I.C.A. sponsored memberships. Registration at the 1959 meetings was 1,292.

"Subscriptions to the Journal of Animal Science increased by 156 to a total of 1605. Of these, 600 go to the United States and Canada, and 1005 go to foreign countries.

"There were 265 papers accepted for presentation and 49 to be read by title for the 1960 program. In addition, there were 8 invitational papers or addresses. The following half-day sessions were planned by program committees: Breeding and Genetics—3; Extension—2; Teaching—1; Meats—4; Nutrition—10; Pasture and Forage—3; Physiology—6. This represented a total of 29 sessions as compared with 25 last year.

"The Secretary appreciates the considerate cooperation and helpfulness of the officers and members. Appreciation especially is expressed to Mrs. Zola Terrill for her assistance in this office."

C. E. Terrill moved acceptance, W. J. Loeffel seconded, carried.

The Treasurer's report was read by C. E. Terrill as follows:

"Expenditures in 1960 were $38,400.66 with an excess of income over expenditures of $3,550.65.

"As of October 31, 1960, the American Society of Animal Production had total assets of $48,840.55 with an increase of $3,550.65.

"The balance in the Morrison Award fund was $4,902.70, leaving $43,937.85 in the General Society Fund."

C. E. Terrill moved acceptance, W. W. Green seconded, carried.

Excerpts of the Auditor's report were read by J. K. Loosli and the suggestion was made that those of the membership who wished to read the report in detail were invited to do so. J. K. Loosli moved acceptance of the Auditor's report, G. L. Robertson seconded, carried.

The Business Manager's report was read by C. E. Terrill as follows:

"Three thousand and nine hundred journals were printed quarterly with an average total cost of $1.93 per copy. A total of 3,774 copies are being mailed to members and subscribers.

"The records of the Business Manager list the fol-

lowing back numbers of the Journal and Proceedings available for sale:

Year	Volume	Number of Copies No. 1	No. 2	No. 3	No. 4
1943	2				
1950	9			24	26
1951	10		69		14
1952	11			45	102
1953	12	11	46	33	
1954	13	221	244	256	336
1955	14	28	38	40	6
1956	15		36	67	392
1957	16	126	124	142	130
1958	17	197	64	88	107
1959	18			32	179
1960	19	178	116	201	180

Proceedings of the Society (Year and Number of Copies)

1915–16	36	1929	18	1934	12
1922	50	1930	19	1935	17
1923	42	1931	48	1940	31
1925–26	30	1932	7		
1927	42	1933	26		

Reprint of Review Papers

1958 Vol. 17 #4 (48 copies)

"These back issues may be purchased from the Business Manager at the rate of $2.25 per number for delivery in the United States and Canada, and $2.50 for other countries. Proceedings, $1.00 each. Reprints of review papers, $2.00 each. Additional back numbers become available from time to time and inquiries should be sent to the Business Manager regarding back numbers wanted which are not listed above. Back numbers in good condition which members wish to dispose of will be purchased for $1.00 per number by the Business Manager."

C. E. Terrill moved acceptance, L. E. Kunkle seconded, carried.

W. D. Gallup read the Editor's report as follows:

"This year (1960) the Journal published 151 research papers and 390 abstracts. The total number of pages was 1366, the largest number in any volume except volume 18 (1959) issued after our 50th anniversary year.

"Rules for the new Distinguished Teacher Award were published in the February issue. The first recipient of this award and the first recipient of the Extension Award were announced with appropriate citations and photographs in the February issue. Abstracts of papers presented at the Southern and Western sectional meetings were published in the May and August issues. These abstracts are identified by an asterisk in the annual index published in the November issue. North Atlantic sectional meeting abstracts will be published in the February issue of 1961.

"During the 12-month period ending November 15, 1960, a total of 202 manuscripts were received for publication. Seventy-four of these have been published, 39 are in press, 32 have been withdrawn or found unacceptable, 22 are being revised by authors and 35 are being reviewed. The editorial board was enlarged to 15 members, and, through the year, an increased number of members (47) not on the board were requested to review papers in their particular field of specialization.

"Reviewers whose terms on the board expire at the end of the year (1960) are L. J. Bratzler, G. P. Lofgreen and R. W. Swift. Their valued counsel and their special contributions on the board are gratefully acknowledged. A. B. Chapman, your very capable associate editor, becomes editor of the Journal, January 1.

"As editor for the past 3 years, I gladly acknowledge with thanks the generous help received from previous editors, from individual members of the editorial board and from those persons who so willingly reviewed manuscripts. And, it is with particular pleasure that I acknowledge the cooperation received from authors in making manuscript corrections and alterations to meet review criticisms. As in the past, the quality of our Journal will continue to be measured in terms of the authors' contributions."

W. D. Gallup moved acceptance, H. H. Stonaker seconded, carried.

The report of the Nominating Committee consisting of W. M. Beeson, R. F. Cox and B. L. Southwell was read by W. M. Beeson nominating H. H. Stonaker for President, T. J. Cunha, University of Florida and C. S. Hobbs, University of Tennessee for Vice President and C. E. Terrill for Secretary-Treasurer. Beeson moved acceptance of report and election by acclamation of H. H. Stonaker as President and C. E. Terrill as Secretary-Treasurer, seconded by J. C. Miller, carried. J. K. Loosli appointed C. M. Kincaid, L. E. Harris and G. L. Robertson as tellers.

C. F. Neumann read the report of the Information Committee as follows:

"At the 52nd annual meeting of the American Society of Animal Production held in the Sherman Hotel, Chicago, Illinois, November 25–26, 1960, 638 animal scientists reported results of experiments in 332 scientific papers delivered to the more than 2000 in attendance from land grant experiment stations, industrial research laboratories and farms, and some doing similar work in foreign countries.

"Besides the many strictly laboratory experiments reported, there were 104 feed lot experiments with 36,370 cattle involved in the experiments. The scientists studied 4990 hogs in 58 experiments reported. And surprisingly, experiments with sheep were more numerous than those with hogs, there being 72 experiments reported, and 7435 sheep involved.

"This is probably due to the ever increasing number of digestive experiments conducted, in which studies are made of the digestive action within the rumen of live animals. Scientists are looking inside the stomachs of live animals, as well as recording, as formerly, the exterior physical characters of the experimental animals, to determine comparative values of feeds in varying rations.

"To give some idea of the magnitude of animal science research projects, the following figures have been compiled.

"There were 234 experiments using 48,795 cattle,

sheep, hogs, and goats. Length of the testing periods varied from a few days to years. A conservative estimate of time put in by each tested group would be 90 days.

"Figuring 90 days of study on each of the 48,795 animals, the total amount of time consumed in the investigations conducted during the past year would be equivalent to 12,032 years of research if the work had been carried out consecutively as one project rather than separately as 234 projects.

"Or, going the other way, since the animals were used 24 hours a day, for 4,391,550 days, multiplication shows that they worked giving their lives to science, for 105,397,200 hours. To stagger the imagination, multiply that by 3600, the number of seconds in an hour.!!

"The work of the American Society of Animal Production, primarily related to last year's annual meeting, was covered in at least 44 articles in 37 daily and weekly newspapers during the past year. We say at least because we have actually seen the clippings from these 37 newspapers. There were likely many more which were not brought to our attention.

"Just to give you some idea of the distribution of the stories here are a few of the papers: Ft. Smith, Arkansas, Times-Record; Chicago, Ill., Tribune; Chicago, Ill., Daily News; Salt Lake City, Utah, Deseret News; Oswego, New York, Palladium Times; Fort Worth, Texas, Star-Telegram; Cedar Rapids, Iowa, Gazette; Hobbs, N. M., News and Sun; Athens, Ga., Banner-Herald and Madison, Wisc., State Journal.

"We also know of at least 104 articles carried in the magazines and newspapers of the livestock industry and meat trade. This amounted to nearly 1,000 column inches of space in such publications as the Corn Belt Dailies, Breeders Gazette, Feedstuffs, National Provisioner, Butchers Advocate, The Cattleman, Farmer Stockman, National Wool Grower, National Hog Farmer, Prairie Farmer, Record Stockman and just about all of the breed publications."

C. F. Neumann moved acceptance, W. M. Beeson seconded, carried.

O. D. Butler read the following report and recommendations of the Research Committee:

"1. After further consideration and discussion with some members of the Society, the Research Committee wishes to repeat the recommendation made in 1959 that consideration be given to the regular inclusion of a greater number of symposia in the Annual Meeting Programs.

"2. A formal request was received from Mr. Farrington R. Carpenter, President, Performance Registry International:

"This committee recommends to the Executive Committee the establishment of a 'task force' composed of representatives of the Beef Carcass Evaluation Committee of the Reciprocal Meats Conference and of appropriate beef cattle production and selection, representatives to be charged with the responsibility of developing certification standards for superior beef animals.

"3. This committee recommends that the Executive Committee appoint a 'task force' to modernize and clarify the nutritive requirements of beef cattle under various environmental conditions and production levels.

"4. Every research station distributes mimeographed progress reports, field day reports, etc., reporting current information which frequently does not warrant publication as a technical paper or bulletin. Routine swapping of such reports seems desirable for wider dissemination of research ideas and current results.

"This committee recommends that the Executive Committee establish and maintain a current mailing list and encourage routine circulation of such material to department heads and directors of research groups. Staff members could obtain additional copies, if desired, from the publishing agency."

C. E. Terrill read the following report of the Monograph Committee:

"During the past year the final revisions were added to the Monograph, 'Techniques and Procedures in Animal Production Research,' and it was edited by Dr. A. B. Chapman. Copies were mailed free to all members just previous to this meeting. Copies may be purchased for $4.00 each to the United States and Canada, and for $4.25 each to all other countries. Thirty-nine hundred copies were printed with the privilege of reprinting within one year. Thanks are due to all those who contributed to the Monograph. The Monograph Committee should be terminated with the acceptance of this report."

C. E. Terrill moved acceptance of the report. W. A. Craft seconded, carried.

G. L. Robertson read the report of the Teaching Committee:

"Because teaching section meetings have been poorly attended in the past, the committee's first move toward developing a 1960 teaching program was to investigate the possibilities of a general program on teaching. We contacted the previous chairman and holdover committee members concerning the reaction of Society members to the 1959 banquet speaker who carried the teaching emphasis of last year's program. Each one was of the opinion that a similar effort should be made this year in an attempt to highlight teaching in a large meeting of the Society membership.

"Upon inquiry to President Loosli, however, the committee was told: 'At the last session of the Executive Committee's meeting last November it was decided that there should be no main banquet speaker next year because the program and presentation of awards took so much time that it made the meeting too long. . . . It was also decided that a general speaker should be brought in for the 11 o'clock session Friday morning. . . . The Executive Committee decided that this program should not be devoted to teaching this year. . . . We recognize that the teaching committee has a real problem because of poor attendance at the evening sessions in the past. It was not thought wise, however, to put major emphasis on teaching two successive years.'

"Being denied the opportunity to develop a program for the general session, the committee then applied itself to the task of arranging a strong teaching section program. The entire work of the committee in arranging this year's program has been accomplished by mail and telephone. Following several exchanges of correspondence in which several suggestions were considered, the committee recommended that Professor T. H. Knox, Head, Animal Husbandry Department, New Mexico State University, and recipient of

the Society's first Distinguished Teacher Award, and Dr. Roy M. Kottman, Dean of Agriculture at Ohio State University make up a panel to discuss the subject of improving college teaching in Animal Husbandry. Their prompt acceptance along with the agreement of Professor George Litton of Virginia to serve as Moderator finalized our teaching section in the form it now appears in your printed program. We especially invite your attendance in the Bal Tabarin room at 9:00 a.m. tomorrow morning. (This teaching section was well attended—about 80 persons were present. Both papers were well received and a lively discussion followed each paper. Many of those present expressed a desire to the Chairman that similar sessions should continue to be held.)

"During the course of the year, the committee received from its own Membership and other Society members several suggestions for focusing interest in the area of teaching. One suggestion was that the teaching section be dropped from the annual meeting and be replaced by a summer workshop for animal husbandry teachers. (It was pointed out that such a workshop would not be in competition with research sessions which undergraduate teachers need to attend.) Another suggestion called for the Society to prepare and distribute a brochure pointing out the opportunities in animal husbandry, as a means of offsetting the declining enrollments in agriculture across the country.

"These suggestions were considered briefly by correspondence during the year and in some detail at a committee meeting this morning. Concerning these suggestions the committee recommends to the Society that

"1. The annual teaching section be continued with even greater emphasis.

"2. In lieu of summer workshops, the committee recommends that the Executive Committee of the Society encourage each Regional Executive Committee to establish an active teaching group to conduct teaching sessions as a part of each regional meeting.

"3. Concerning the brochure, the Committee recommends no action by the Society at this time, but suggests that subsequent Teaching Committees give further serious consideration to this matter."

G. L. Robertson moved acceptance of the report, C. M. Kincaid, seconded, carried.

The Extension Committee was active during the year in initiating 11 committees to improve livestock extension work but no formal report was made.

The Membership Committee report was given by H. H. Stonaker. All Animal Husbandry Departments were contacted during the year and a net gain of 219 members was recorded for 1960.

H. H. Stonaker moved acceptance, O. F. Pahnish seconded, carried.

The Committee of Tellers reported the election of T. J. Cunha as the new Vice President of the Society.

The report of the Necrology Committee prepared by W. D. Salmon was presented by J. K. Loosli. The report was approved for publication in the Journal of Animal Science. A moment of silent tribute was observed in honor of the deceased members.

Dr. Loosli introduced the new Editor, Dr. A. B. Chapman of the University of Wisconsin. He also announced the decision of the Executive Committee to change the page size of the Journal of Animal Science to 7" x 10" with a two column format. This will necessitate an increase in the reprint charge per page but the number of pages will be less.

Dr. Loosli reported that the membership would be polled in 1961 as to the time and place of the annual meeting. It seemed probable that a change could not be made before 1963.

Report of Special Committees

T. C. Byerly, American Association for the Advancement of Science Representative, presented the following report:

"Section O, Agriculture conducted an outstanding symposium on Germ Plasm at the 1959 meeting of the A.A.A.S. The symposium was organized by Ralph Hodgson, a member of this Society, and several other Society members also made outstanding contributions to it.

"This year's symposium at the New York meeting starting on December 26, 1960, has been organized by Dr. Firman Bear, long a distinguished professor of soil science at Rutgers, the State University of New Jersey. The symposium will deal with problems of urbanization, a topic of broad general interest.

"The A.A.A.S. has during 1960 extended its objective covering of pending legislation of interest to science in its journal, Science, and has energetically increased its activities in the fields of science and education and the role of science in society."

T. C. Byerly moved acceptance, R. E. Comstock, seconded, carried.

The report of the Committee on Intersociety Cooperation and Scientific Manpower Commission was prepared by J. E. Foster but was not read. C. F. Sierk moved acceptance, W. Gifford seconded, carried. The report is as follows:

"The sixth Annual Report was presented at the business meeting of the American Society of Animal Production last year. Since then, S.M.C. meetings have been held in Washington, D. C., on February 2, May 10 and November 16. The Committee on Intersociety Cooperation met in Washington on October 4 to review its accomplishments and to discuss possible means of future service to the organizations. At this time, Mr. John W. Barnard of the U. S. Department of Agriculture Soil Conservation Service, Washington, D. C., was elected Executive Secretary. He succeeded Dr. W. H. Garman of the National Plant Food Institute who had served in this capacity for a

number of years. Your Society made a contribution again this year of $150.00 to the Scientific Manpower Commission.

"At the February meeting of the S.M.C., Dr. Frank P. Cullinan of the Agricultural Research Service, Beltsville, Maryland, was elected President for 1960. The American Institute of Chemists was accepted as the Commission's eleventh nominating agency.

"The Executive Director, at the February and May meetings, presented summaries of current activities of the Scientific Manpower Commission and copies of his reports are available for the nominating agencies. One of the many items contained in the reports is that the S.M.C. leaflet 'Critical Personnel and the Draft' is now in its second printing (4,000) and is still in active demand. A substantial part of S.M.C. business is concerned with Selective Service and Military problems.

"The Commissioners were requested to again ask their respective societies for contributions to S.M.C.

"At the November meeting of the S.M.C., Dr. Frank P. Cullinan was renominated for President for 1961 and Dr. M. H. Trytten of the National Research Council was nominated for Vice President. Considerable time was devoted to a discussion of a study or conference on Occupational Titles for Agriculture and Dr. Meyerhoff, the Executive Director of S.M.C., was authorized to set up the conference. Following a report and discussion on the demand and supply problems in the medical-biological field (including agriculture), Dr. Meyerhoff was also authorized to investigate the feasibility of a survey on this vital problem and the possible sources for financing it. The next quarterly meeting for S.M.C. was set for February 7, 1961."

C. F. Sierk read his report on the National Research Council as follows:

"1. *Annual Meeting, National Research Council*

"The third annual meeting of the National Research Council took place at the Academy-Research Council, March 24–26, 1960. Following the same general plan as in the two previous annual meetings, plenary sessions were held on Thursday afternoon and evening, and on Saturday morning. Plenary sessions included review of the activities of the Research Council since the last meeting, and panel discussions on three major topics which included:

"(1) Dynamics of National Resources,

"(2) Grants and Fellowships as Factors in the Development of Scientists, and

"(3) Transportation and Patterns of Living.

"At the final plenary session, critical attention was given to the panel discussions as well as summarizing the principal interests and activities of the National Research Council.

"2. *Annual Meeting, Division of Biology and Agriculture,* March 25, 1960

"Thirty-nine societies are now represented in the Division, which is an increase of five over last year. The new societies are:

"American Society of Agricultural Engineers

"Biophysical Society

"Society of Protozoologists

"Radiation Research Society

"American Society of Range Management

"In addition to the society representatives, the membership of the Division consists of nine members-at-large, and thirteen liaison members.

"The annual meeting under the chairmanship of H. Burr Steinbach, except for some opening comments on the activities of the Division the past year, was a very informal type of meeting with no agenda and no oral reporting of activities by the staff of the Division. There was a wide range of ideas and reactions to them. No single topic held the attention of the members long enough to reach any conclusions, but certainly ample opportunity was provided for members to speak on any topic they considered the concern of the Division.

"The one single topic receiving the most attention was the need for constructive action by the Division in the field of biology education.

"3. *Annual Meeting, Agricultural Research Institute*

"The ninth annual meeting of the Agricultural Research Institute was held October 17–18, 1960, at the Academy-Research Council. The program included three excellent panel discussions on the topics:

"(1) The Place of Engineering Research in Plant and Animal Production and Utilization in Our Modern Agriculture,

"(2) The Interresponsibility of Land-Grant Colleges, Government, Industry, and Regulatory Agencies with Respect to Agricultural Research and Interpretation of Results to the Public,

"(3) Research Advances and Problems in the Animal Sciences.

"The governing board of Agricultural Research Institute met on the day preceding the annual institute meeting. The institute is faced with a very real problem in increasing the Class A membership so that the work of the Agricultural Board can be adequately supported. The proceedings of the ninth annual meeting which will be available from the National Academy of Sciences, National Research Council, will be of real interest to members of this society.

"4. *Division Publications*

"A major activity of the Division of Biology and Agriculture is the publication of the efforts of the numerous committees. A recent summary of publications available through the Printing and Publishing Office, NAS-NRC, lists the titles of 64 publications. In addition to these Division publications, no less than 20 books (published by commercial publishers) have resulted from Division sponsored conferences or symposia or from staff work.

"5. *International Congress of Zoology*

"The 16th International Congress of Zoology will be held in Washington, D. C., August 21–27, 1963. Preparations for the Congress will be made by the Division of Biology and Agriculture. Dr. G. B. Moment, Professor of Biology, Goucher College, will serve as Secretary-General of the Congress."

C. F. Sierk moved acceptance, E. H. Vernon seconded, carried.

In the absence of N. R. Joshi, representative for the European Association for Animal Production, W. J. Loeffel moved acceptance, J. C. Miller seconded, carried. The report is as follows:

"The VIIth Study Meeting of The European Association for Animal Production was held in Stockholm from 7–11 June 1960.

"The main topic for presentation of papers for discussions was research work with monozygotic

cattle twins. There were about 12 papers presented for discussion.

"The opening paper was presented by Professor A. Hansson and Dr. Claesson and provided an excellent review of the use of zygotic twins in animal experimentation. The experience of many countries in the diagnosis frequently and the collection of twins was essentially similar. Conclusions emerging from twin experiments replicated in various countries also appeared similar with the exception of plane of rearing experiments. In this field of work the experimental results of New Zealand research are contrary to the results of work at Wiad and the Rowatt Research Institute.

"The more interesting points arising from the various papers may be listed as follows:

"1. Blood grouping is now an established adjunct of monozygotic twin diagnosis.

"2. Many twin experiments are still designed using far too few monozygotic twins.

"3. The value of dizygotic twins for growth studies has not been appreciated.

"4. The demonstration that genetic differences in milk yield between dairy herds in New Zealand are negligible raises the question of the extent to which this conclusion may be generalized for other countries. This becomes important, particularly in view of the fact that the designs of successful breeding programs are directly dependent upon knowledge of genetic differences between herds.

"The various Study Commissions also had short sessions.

"Commission on Animal Genetics

"The Commission on Animal Genetics discussed a paper by Dr. Bowman of the United Kingdom on the use of heterosis in livestock feeding programs. Dr. Bowman concluded his paper by mentioning that in all probability the best practical use of heterosis may well be achieved by selecting for one, or perhaps two, traits in each breeding group and then crossing groups in desired combinations so that the final crossbred group shows heterosis in total productivity.

"There were also several papers dealing with crossbreeding experiments in cattle, pigs and poultry.

"The Study Group also discussed many papers on the genetics of the bio-chemical polymorphisms.

"Dr. J. Rendel (Sweden) presented a paper reviewing the blood groups in cattle and poultry. He also presented some evidence in both species of a relationship of certain blood groups and production traits.

"Dr. Neimann-Sorenson and Dr. Alan Robertson presented a paper on the relationship between the blood groups and production traits from a large quantity of data obtained from the Danish progeny testing stations. Certain blood groups exhibit a significant effect on fat test, but only a small fraction of the total variation in fat test could be accounted for by the blood-group yields.

"Dr. Robertson further reviewed the numerous polymorphic situations arising in other body constituents.

"Commission on Pig Production

"The subject of discussion was performance testing in pigs. Dr. Smith of Edinburgh presented a paper outlining an excellent case for performance testing. Problems relating to genotype in the environmental interactions were also discussed.

"The Commission on Pig Production is also finalizing its presentation on feeding standards for pigs.

"There was also some discussion on the use of artificial insemination in pigs.

"Commission on Sheep and Goat Production

"Discussions took place on the planned Meeting of Experts organized by FAC and EAAP on Production and Utilization of Sheep and Goat Milk in the Mediterranean Region to be held in Athens from 17–22 October 1960.

"During the year under report a meeting was held in Brussels (21–22 March) dealing with the Belgian Pietrain breed of pig, as this breed is attracting a good deal of attention all over Europe.

"A meeting will be held in London during 1961 for standardizing dissection methods in pigs."

C. E. Terrill moved acceptance of the Ralston Purina Award Committee report by L. E. Hanson without reading, A. L. Harvey seconded, carried. The report is as follows:

"The Ralston Purina Fellowship Awards Committee met in St. Louis, March 28, 1960. The Committee was composed of five persons: R. H. Thayer, Oklahoma State University, representing the Poultry Science Association; N. L. Jacobson, Iowa State University, representing the Dairy Science Association; Robert MacVicar, Oklahoma State University, representing the American Association of Land Grant Colleges and State Universities; W. A. Hagan, ARS Animal Disease Laboratory, Ames, Iowa, representing the American Veterinary Medical Association; and L. E. Hanson, University of Minnesota, representing the American Society of Animal Production.

"Thirty-seven applications were reviewed by the Committee, which was the same number received the previous year. The average apparent quality of the applicants was of a very high order with many near perfect scholastic records. Awards were made on the basis of scholarship, undergraduate program, proposed research, recommendations and other pertinent data. One award was made in each of the fields of Poultry Husbandry and Veterinary Medicine; five awards were made in Animal Husbandry; and three awards were made in Dairy Husbandry.

"The names of the awardees were forwarded to Dr. W. M. Beeson, former President of the Society, by J. D. Svkes, Ralston Purina Company on March 30, 1960. The Ralston Purina Company is to be commended for making these fellowships available."

The report of the Resolutions Committee was given by W. Gifford as follows:

"The Resolutions Committee offers the following:

"(1) Be it resolved that a letter be sent to Hotel Sherman expressing appreciation of the Society for facilities, the assistance and many courtesies shown by the hotel management during the 52nd Annual Meeting.

"(2). Be it resolved, that the Society express its appreciation to the Committee, authors and the editor for their efforts in preparation of a much needed monograph. This monograph entitled, 'Techniques and Procedures in Animal Production Research' just published by the Society. The various sections were written by C. H. Henderson, Gordon E. Dickerson, L. E. Casida, E. W. Crampton, Ivan L. Lindahl, O. G. Hankins, et al., and edited by A. B. Chapman.

"(3). Be it resolved that the Society go on record, commending Dr. W. D. Gallup for his faithful and competent service to the Society as Editor of the Journal of Animal Science.

"(4). Be it resolved that a letter be sent by the Secretary expressing appreciation of the Society to the donors of

"The Morrison Award

"American Feed Manufacturers Association Award

"Extension Animal Husbandmen Award
(Courtesy of Chas. F. Pfeizer and Co.)

"Distinguished Teaching Award
(Courtesy of Swift and Co.)

"for their support which serves to encourage and honor meritorious service.

"(5). Be it resolved that we express to the officers and committees of this Society our thanks for their time and faithful service in our behalf during this year.

"(6). H. W. Norton offered a resolution from the floor of appreciation for the arrangement and manner of presentation of the program this year."

W. Gifford moved acceptance of the report, O. G. Bentley seconded, carried.

Meeting adjourned at 9:45 p.m.

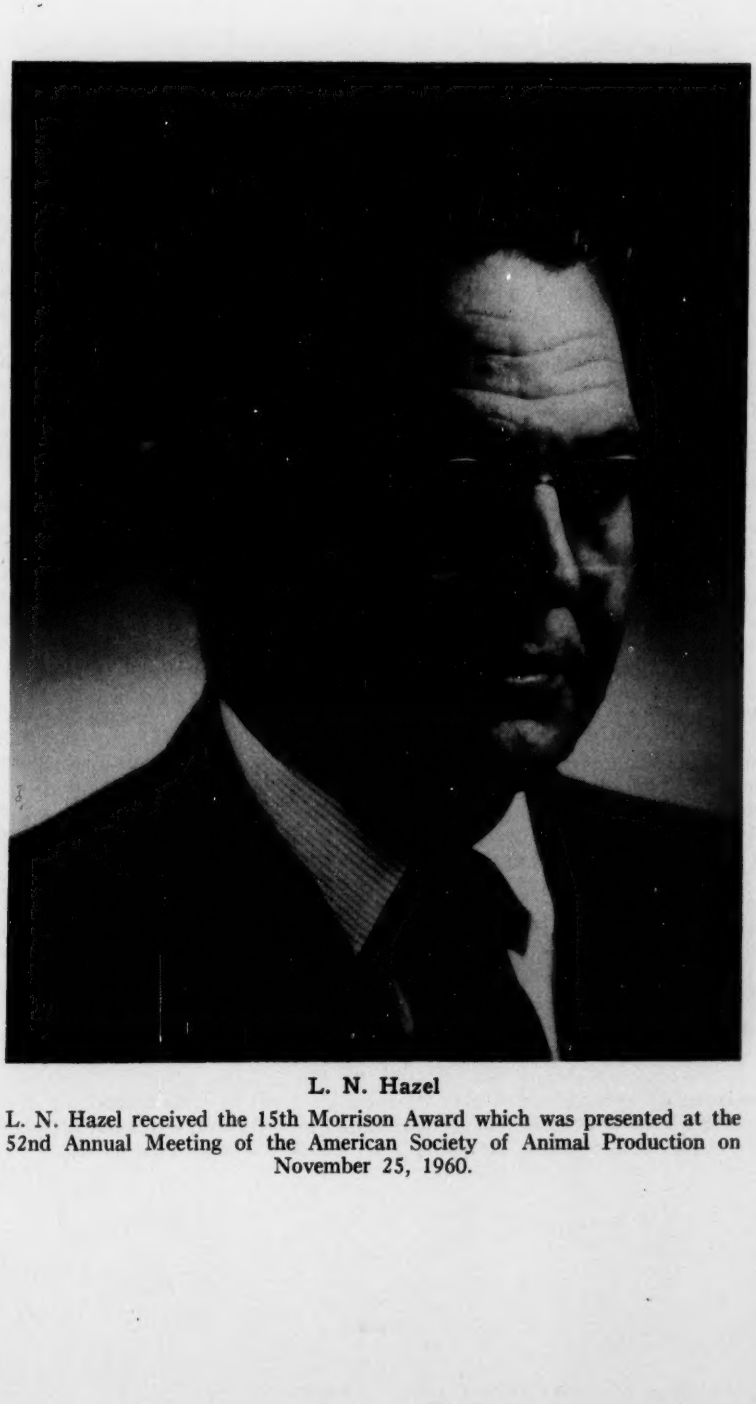
THE MORRISON AWARD

1960

Lanoy Nelson Hazel was born in Shannon Clay in Texas on April 23, 1911. He grew up in the small Texas town of Spur which was the headquarters of one division of the famous SMS ranch. Even as a boy, he was actively interested and a part-time worker in the operations of this ranch and of the other livestock interests around the town. He took his B.S. degree in Animal Husbandry at Texas Tech in 1933, his M.S. degree in Genetics at Texas A & M in 1938, and his Ph.D. degree in Animal Breeding at Iowa State in 1941. Between 1933 and 1938 he worked at various livestock and farming enterprises in Texas and Florida.

After receiving his Ph.D. degree he conducted research for a year and a half on swine breeding at the North Platte substation of the University of Nebraska, three years of research at the U. S. Sheep Breeding Station at Dubois, Idaho, and more than a year of research for the Kimber Poultry Breeding Farms at Niles, California. Since early 1947 he has been Professor of Animal Breeding at Iowa State University where he is responsible for the undergraduate and much of the graduate teaching in this field and for the research on breeding swine and beef cattle.

He is the author or co-author of some fifty scientific articles and bulletins, besides numerous articles for the popular press. Also he has helped many colleagues unselfishly in interpreting and publishing their findings. More than any other one person, he is responsible for spreading the idea of selection indexes among animal husbandmen and expanding and refining that to make the indexes fit a wide variety of practical situations. Out of this came much of what is thus far known about the distinction between phenotypic and genetic correlations and how to cope with genetic antagonisms. He pioneered the use of the mechanical probe for measuring thickness of backfat on live pigs and he has been a valued counsellor and booster in developing the boar-testing stations. He has had a prominent part in adapting modern statistical methods to practical use for animal husbandmen in reaching more nearly complete solutions to their problems.



L. N. Hazel

L. N. Hazel received the 15th Morrison Award which was presented at the 52nd Annual Meeting of the American Society of Animal Production on November 25, 1960.

AMERICAN FEED MANUFACTURERS' ASSOCIATION AWARD

1960

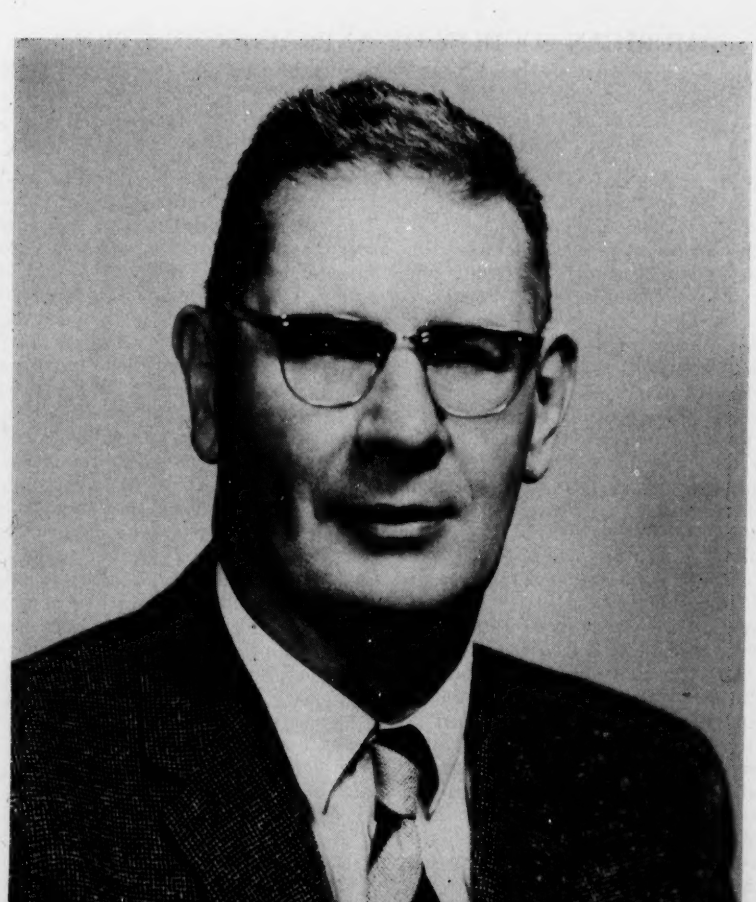
B. Connor Johnson, the recipient of the 1960 American Feed Manufacturers' Association Award, has contributed much to our basic and fundamental knowledge of nutrition.

During the years of 1957, 1958 and 1959 Dr. Johnson was the author or co-author of thirty-seven scientific papers published in eight scientific journals in this country and several foreign journals. Dr. Johnson has been very versatile in his research efforts, having studied the nutritional status of many species of animals; he has been active in many specialized areas of nutrition and biochemistry and his research findings have contributed much to our present knowledge of the nutrition and physiology of animals including man. His interests have embraced studies of nitrogen sources for sheep, vitamin deficiencies of calves, vitamin B_{12} metabolism and function, metabolism of radioactive vitamin A, radiation sterilization of foods, nutritional role of antioxidants and other chemical compounds, effect of antibiotics on the intestinal synthesis of vitamins, and amino acid metabolism and requirements of various species of animals.

In 1956 Dr. Johnson was selected as a member of the President's Second Atoms for Peace Mission to South America. For a period of six months in 1958 he served as International Cooperation Administration consultant to the Organization for European Economic Cooperation on application of atomic energy to animal science. Dr. Johnson was a United States Delegate to the 2nd United Nation Conference on peaceful uses of atomic energy held at Geneva, Switzerland in 1958. He has presented on invitation research papers at many national and international meetings, most noteworthy of the international conferences being the 3rd International Vitamin Symposium at Poznan, Poland in 1959 and the International Symposium on Vitamin A and Metabolism at Bürgenstock, Switzerland in 1960.

Dr. Johnson was born in Regina, Saskatchewan, Canada, April 28, 1911. He was graduated with the B.A. degree in organic chemistry from McMaster University, Ontario, Canada in 1933, and one year later was awarded the M.A. degree by that institution. During the following three years he was employed by Canadian Canner's Ltd. at Hamilton, Ontario.

In 1940 the University of Wisconsin awarded him the Ph.D. degree in biochemistry. From 1940 to 1942 he served as a postdoctoral fellow in the Department of Animal Science at the University of Illinois and subsequently took a position for one year as a research biochemist with the Golden State Co., Ltd., in San Francisco, California. In 1942 he returned to the University of Illinois and has been a member of the staff of the Department of Animal Science since that date.



B. C. Johnson

B. C. Johnson received the 13th American Feed Manufacturers' Association Award which was presented during the 52nd Annual Meeting of the American Society of Animal Production in Chicago on November 25, 1960.

AMERICAN SOCIETY OF ANIMAL PRODUCTION EXTENSION AWARD

1960

Wilbur H. Bruner was born October 17, 1905, on a farm in Fulton County, Ohio. He graduated from Delta High School in 1923, received his Bachelor of Science degree from Ohio State University in 1927, and did graduate work at Cornell and Ohio State Universities.

He was appointed County Agricultural Agent for Preble County in 1936, and served in this capacity until 1947. During his work with Preble County swine producers, he developed the pilot program that provided the basis for the Ohio Swine Improvement Program which now distinguishes his career as an Extension Specialist.

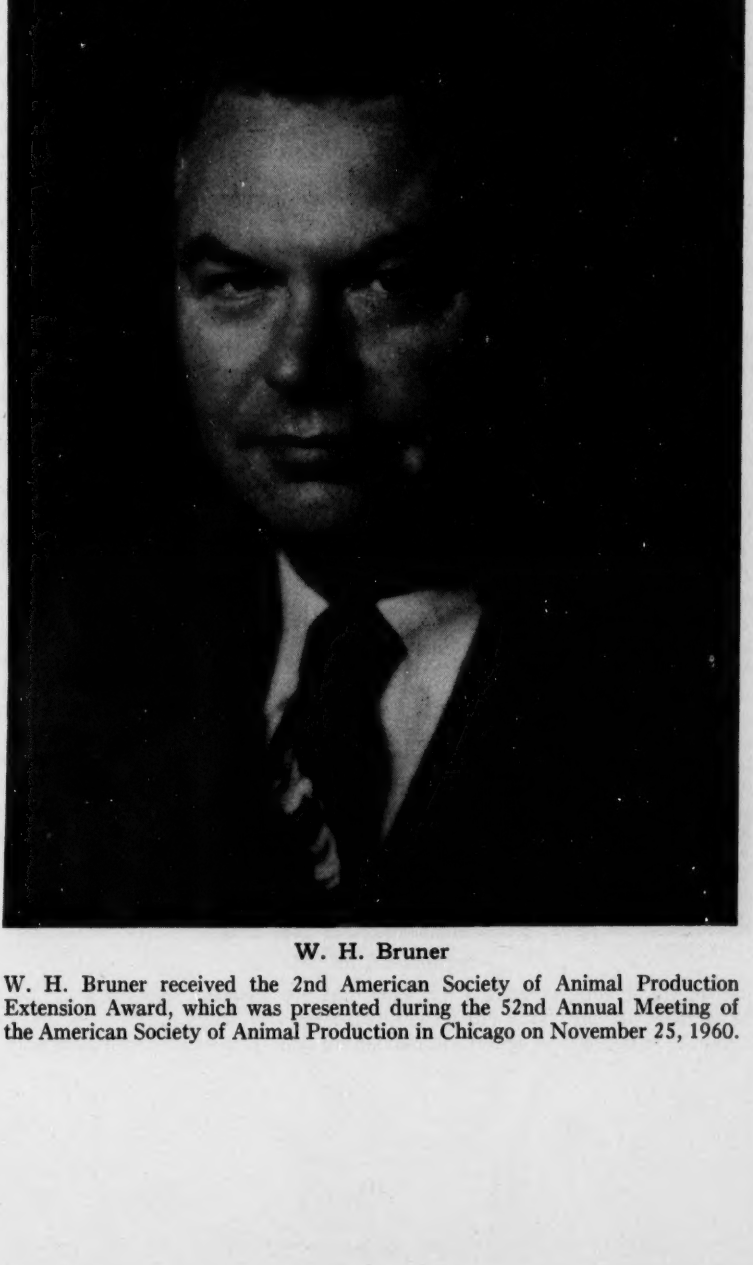
In August, 1947, the Ohio Wool Growers Cooperative Association invited Mr. Bruner to serve as their Secretary and Manager. He resigned from the Extension Service and served this organization for two years.

In 1949, the Agricultural Extension Service at Ohio State University initiated a project in Swine Marketing Improvement under the Research and Marketing Act. Mr. Bruner returned to the Extension Service as Swine Marketing Specialist to lead this project at a reduction in salary from his commercial position, because of the challenge that lay in the development of a differential pricing system for meat-type hogs. He served in this capacity until June, 1954, when the Ohio State University provided a Swine Evaluation Station with capacity for 108 pairs of pigs. At this time, his title was changed to Extension Specialist in Animal Science and he was given the assignment of leading the Ohio Swine Improvement Program. He continues to serve in this capacity.

Mr. Bruner's career as an Extension Specialist has been marked by four traits: his initiative; his acceptance of a challenging situation; the friendly and convincing manner in which he secures the support of individuals and swine organizations for the Ohio Swine Improvement Program; and the determined and thorough manner in which he carries out his program.

Mr. Bruner's influence on swine breeders in Ohio is remarkable when it is noted that more than half of the breeders have participated in his program within a period of six years, and at a rate that is limited only by the capacity of the Swine Evaluation Station. Because of his status as a pioneer in swine improvement, his counsel has been sought by organizations in other states who wish to start similar programs. He has assisted in planning the swine improvement programs functioning in Michigan and Indiana. He has been called to Georgia, North Carolina, Tennessee, and New York to explain the Ohio Swine Improvement Program. His work and records have produced data proving that meat hogs gain more efficiently than fatter kinds. His analysis of his testing data has established characteristic differences between barrows and gilts regarding rate of gain and carcass quality. He has written, or assisted in writing, five articles that were published by the Journal of Animal Science. His writings also include Ohio Agricultural Experiment Station reports, Agricultural Extension bulletins, mimeographs, and news articles.

Mr. Bruner's skill in transforming the unorganized and uninformed into cohesive functioning groups, that have direction and purpose, illustrates leadership of the highest order. He has initiated and enriched an active extension educational program in swine improvement by his honest brand of leadership and by his great influence on the attitudes and practices of the swine producers. The enduring nature of his program is testimony of how well he has built.



W. H. Bruner

W. H. Bruner received the 2nd American Society of Animal Production Extension Award, which was presented during the 52nd Annual Meeting of the American Society of Animal Production in Chicago on November 25, 1960.

AMERICAN SOCIETY OF ANIMAL PRODUCTION DISTINGUISHED TEACHER AWARD

1960

Marion Eugene Ensminger is an outstanding teacher of young people as well as adults.

He was born near Stover, Missouri, May 28, 1908. After growing up on a dairy and livestock farm and participating in F.F.A. and 4-H Club work, he earned his B.S. and M.S. degrees at the University of Missouri in 1931 and 1932, respectively. The University of Minnesota granted him a Ph.D. degree in 1942. Dr. Ensminger married Audrey Helen Watts of Winnipeg, Canada, in 1941; and they have one son, John.

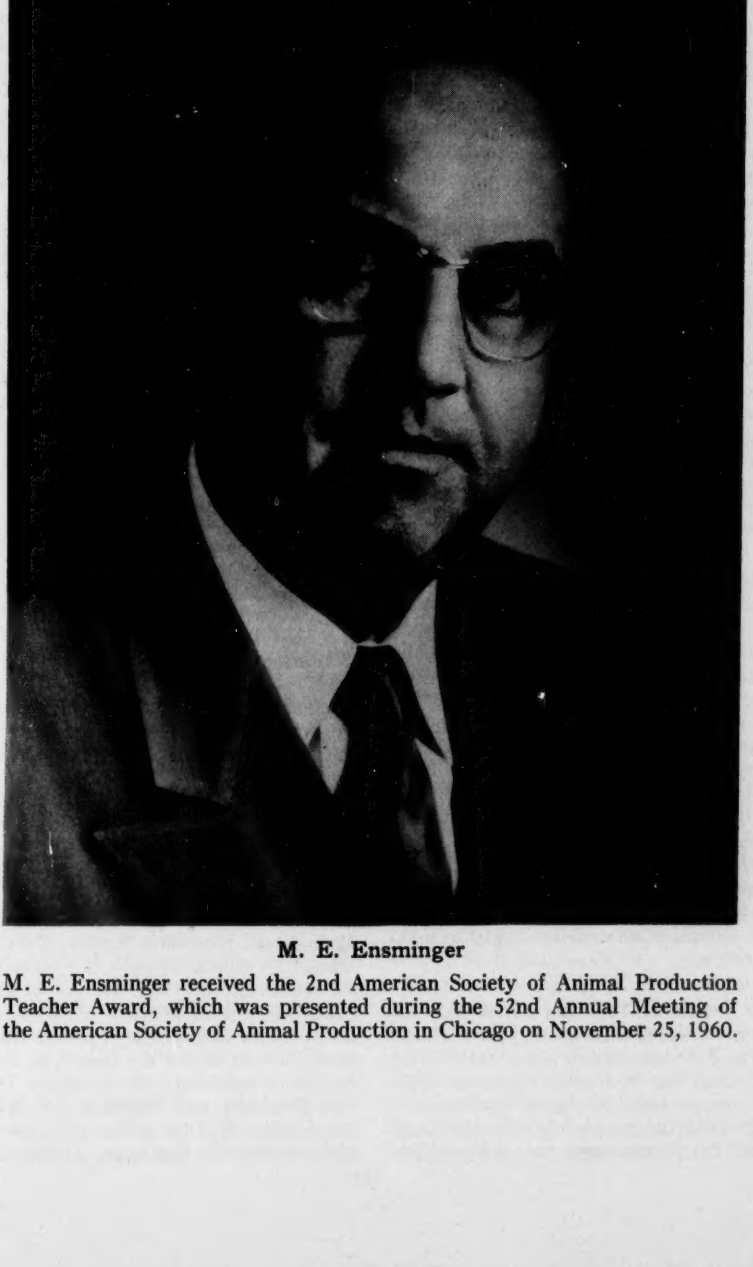
"Dr. E.", as he is called by his students, taught at the State Teachers College, Maryville, Missouri, during the summers of 1931 and 1932. The year 1934 was spent as a Soil Conservation Specialist and from 1934 to 1937 he managed the Dixon Spring Project, Robbs, Illinois. The following three years, Dr. Ensminger taught at the University of Massachusetts, leaving there in 1940 to accept the position of Professor and Chairman of the Department of Animal Science at the Washington State University.

Dr. Ensminger has been honored by membership in Alpha Zeta, Lambda Gamma Delta, Sigma Xi, A.A.A.S., and was elected to the Washington State University Student's Hall of Fame in 1958. He is a member of a number of professional societies. After serving as Secretary-Treasurer, 1956–57, and Vice President, 1957–58, he was President of the Western Section of the American Society of Animal Production and served on the Executive Committee of A.S.A.P. during 1958–59.

Dr. Ensminger is an outstanding classroom lecturer, presenting well-organized and challenging courses in a factual yet dynamic way. His own wide interests and knowledge, and ability to translate research into layman's language, result in an enrichment and broadening of the students' viewpoint and stimulate them to hard work and high personal standards. Dr. Ensminger takes an unusual interest in the welfare of his students and frequently counsels them long after graduation.

Dr. Ensminger's influence extends far beyond the lecture room. He has written 6 widely used textbooks; has been an author or co-author of approximately 300 popular and technical articles, including the U.S.D.A. Farmer's Bulletin, "Light Horses", of which 150,000 were printed for the first run. His column, "The Stockman's Guide", appears in 15 state and regional magazines. In the area of adult education, in addition to responding to numerous requests for talks, he has initiated a series of unique educational events at W.S.U., such as the Stockmen's Short Course, feeder days for all classes of stock, and the Light Horse Judging School and Show. These events attract about 7,500 persons yearly from many states and foreign countries.

In addition to his many duties as a teacher and administrator, Dr. Ensminger has acted as a consultant for the Atomic Energy Commission and is presently on the Board of Field Advisors to the Small Business Administration, Washington, D. C.



M. E. Ensminger

M. E. Ensminger received the 2nd American Society of Animal Production Teacher Award, which was presented during the 52nd Annual Meeting of the American Society of Animal Production in Chicago on November 25, 1960.

FELLOWS OF THE AMERICAN SOCIETY OF ANIMAL PRODUCTION

In recognition of distinguished service over a period of 25 years or more, the title of Fellow has been conferred upon the following men:

THEODORE CARROLL BYERLY. Dr. Byerly was born at Melbourne, Iowa, on May 3, 1902. He received three degrees from Iowa State University; the A.B. in 1923, the M.S. in 1925, and the Ph.D. (in zoology) in 1926. In 1929, he married Miss Helen Frances Freeman; and the Byerlys have three children. He taught zoology at the University of Michigan from 1926–28 and at Hunter College in 1928–29. From 1929–37, he served as a physiologist in the Division of Animal Husbandry, B.A.I., U. S. Department of Agriculture. From 1937–41, he was on the staff of the University of Maryland. He returned to the U. S. Department of Agriculture and served as Head of Poultry Husbandry Investigations from 1941–47; and in 1947 he was made Head of the Animal and Poultry Husbandry Research Branch. He became Assistant Director of Livestock Research in 1953, and in 1957 he was appointed Deputy Administrator of Production Research, Agricultural Research Service, U. S. Department of Agriculture. Dr. Byerly is recognized for his research in embryonic growth, efficiency of feed utilization, and effect of light on reproduction. He is the author or co-author of numerous research articles. In 1943, he received the Borden Award. Currently, he is President of the Poultry Science Association. For several years, he has been the ASAP representative on the AAAS Council. Dr. Byerly has demonstrated that a person well trained in the basic sciences can be successful in many areas. He is known for his keen, analytical mind and his ability to make people think.

EVAN F. FERRIN. Professor Ferrin was born at Marcus, Iowa, on February 9, 1888. He obtained the B.S. degree from Iowa State College in 1911, and the honorary Master of Agriculture degree from the same institution in 1920. In 1912, he married Miss Stella Drennan; and the Ferrins have two children. Professor Ferrin has spent his entire career in college work. In 1912, he served as an Instructor at Texas A & M; in 1912–13, at Iowa State College, starting as an Assistant Professor and advancing to the rank of Associate Professor the second year; in 1918 to 1920, as an Associate Professor at Kansas State College; and from 1920 to his retirement in 1956 at the University of Minnesota, as a Professor, and as Head of the Department of Animal Husbandry from 1949. He has had a long and illustrious career as a teacher, swine scientist, and swine judge; and he pioneered in swine improvement through carcass evaluation and production testing. He is the author of many journal articles, bulletins, and feature articles. Professor Ferrin distinguished himself as one of America's foremost swine authorities and judges, as an able teacher, investigator, and administrator; and, above all, as a scholar and a gentleman.

WILLIAM GARFIELD KAMMLADE. Dr. Kammlade was born at Bangor, Wisconsin, August 11, 1892. He obtained the B.S. degree from the University of Wisconsin in 1915, and the M.S. and Ph.D. degrees from the University of Illinois in 1917 and 1931, respectively. In 1921, he married Mary E. Bell; and the Kammlades have one son. From 1915 to 1937, Dr. Kammlade served the University of Illinois, where he advanced from Assistant to Associate Professor of Animal Husbandry. From 1937 to 1938, he served as Professor of Animal Husbandry, Utah State Agricultural College. In 1939, he returned to the University of Illinois as Professor of Animal Science and Chairman of the Dixon Springs Experiment Station. In 1949, he became Associate Director of the Agricultural Extension Service, University of Illinois, in which capacity he continued until his retirement in 1960. He is the author of numerous journal articles, bulletins, and feature articles and of the textbook *Sheep Science*. He has served the American Society of Animal Production as Secretary-Treasurer, Vice-President, and President. Dr. Kammlade has distinguished himself as a teacher, investigator, sheep husbandman, author, and ad-

ministrator; and he has no peer as a gifted speaker and master of ceremonies—noted for his ready and quick wit.

WILLIAM JOHN LOEFFEL. Professor Loeffel was born in St. Louis, Missouri, on August 31, 1894. He received the B.S. degree from the University of Missouri in 1917, and the M.S. degree from the University of Nebraska in 1929. In 1919, he married Miss Hazel Jane Baer; and the Loeffels have three sons. He served as a Scientific Assistant, B.A.I., in the U. S. Department of Agriculture from 1917 to 1919. In 1919, he joined the Animal Husbandry Department, University of Nebraska, where he subsequently served as an Assistant Professor 1919–25, Associate Professor 1925–36, Professor from 1936 and Chairman of the Department from 1938 until his retirement in 1959. He is the author or co-author of numerous publications. He has served the American Society of Animal Production as Editor of the Proceedings, Secretary-Treasurer, Vice President, and President. Professor Loeffel is recognized as a dedicated and able teacher, investigator, administrator, and public servant. His pioneering of teaching and research in meats has been particularly noteworthy, and he has rendered yeoman's services on many important national and state committees.

HAROLD HANSON MITCHELL. Dr. Mitchell was born at Evanston, Illinois, on January 22, 1886. He received three degrees from the University of Illinois; the B.S. in 1909, and M.S. in 1913, and the Ph.D. (in chemistry) in 1915. In 1910, he married Miss Ethel Opal Kilbury; and the Mitchells have three children. All of his distinguished career has been at the University of Illinois. He served as Assistant Station Chemist from 1911–13, Associate in Animal Nutrition 1913–18, Assistant Professor 1918–20, Associate Professor 1920–25, and Professor from 1925 until his retirement in 1954. Dr. Mitchell is world renowned for his research in metabolism of proteins, energy, and minerals, and in comparative nutrition. He is the author of numerous publications and co-author of *The Biochemistry of the Amino Acids*. In 1945, he received the Borden Award, administered by the American Institute of Nutrition; and, 1950, he received the Morrison Award of the American Society of Animal Production. Dr. Mitchell has made a profound and lasting impression in both animal and human nutrition; as a true scientist and pioneer in animal nutrition, as a teacher of teachers and research workers, and through his reviews or abstracts and publications.

VICTOR ARTHUR RICE. Dr. Rice was born in Cleveland, Ohio, May 4, 1890. He was an honor student at North Carolina State College. He obtained the B.S. degree from North Carolina State College in 1917 and the M.S. degree from Massachusetts State College in 1923. In 1921, he was married to Miss Laura Bussells; and the Rices have two daughters. During his distinguished career at Massachusetts State College, he served successively as Assistant State Club Leader from 1916 to 1919, Assistant Professor of Animal Husbandry from 1919 to 1930, Head of Animal Husbandry from 1930 to 1955, and Dean of the School of Agriculture from 1930 to 1950. He is the author of hundreds of popular articles and the senior author of two widely used books, *Breeding and Improvement of Farm Animals* and *Breeding Better Livestock*. In 1946, North Carolina State College awarded him an honorary Doctor of Agriculture degree. Dr. Rice is an effective administrator, inspiring teacher, talented and popular author and lecturer, and authority on breeding better livestock. He has inspired high standards of performance for those who have studied under or been associated with him.

HERBERT EDWIN ROBINSON. Dr. Robinson was born November 7, 1907, at Henderson, Montana. He obtained the B.S. degree (in chemistry) from the University of Montana, in 1927; and the Ph.D. degree (in nutrition and biochemistry) from the University of Pittsburgh in 1931. In 1955, he took graduate work at the Harvard Business School. In 1934, he married Miss Frances L. Young; and the Robinsons have two children. In 1932, Dr. Robinson joined the Research Laboratories of Swift and Company, Chicago, as Research Nutritionist, in human and animal nutrition; and, in 1939, he also assumed responsibility for directing research in fats and oils. Progressively, he has advanced to more responsible administrative posts with his company. He became Assistant Director of Research in 1941; Assistant Director of Laboratories in 1950; Director of Laboratories in 1953; and Vice President in Charge of Research, his present position, in 1959. He has had much to do with the progressive grant-in-aid and nutrition fellowship programs sponsored by his company;

and he has authored numerous scientific articles. Dr. Robinson—a forceful and dynamic personality—is recognized for both his scientific and administrative abilities, through which he has contributed richly to both human and animal nutrition. His research findings have helped to revolutionize the processing and merchandising of meats.

PHINEAS STEVENS SHEARER. Dr. Shearer was born at Melbourne, Iowa, May 24, 1889. He obtained the B.S. degree from Iowa State University in 1912, and the M.S. in 1928. In 1918, he married Miss Mary C. Kelly; and the Shearers have three children. He served on the Animal Husbandry staff at the University of Nebraska in 1912. He returned to Iowa State University as an Assistant Professor in 1913; he became an Associate Professor in 1914, a Professor in 1919, and Head of the Department of Animal Husbandry in 1935, in which capacity he served until his retirement in 1954. He taught many different courses and coached the livestock judging teams; and he authored or co-authored numerous publications relating to sheep, swine and beef cattle. He served the American Society of Animal Production as Vice President and as President, and in 1953 he was the Honor Guest of the Society. Professor Shearer has contributed richly to the Animal Husbandry field as a teacher, investigator, administrator, and livestock judge. He developed an outstanding Department of Animal Husbandry at Iowa State, where he set lofty goals for all those who follow in his footsteps.

MEMORIALS

Robert H. Black

Robert H. Black, Dean and Director of Agriculture and Home Economics at New Mexico State University, died suddenly at his home in Mesilla Park, N. M., April 12, 1960.

He was born in Noble, Oklahoma, October 28, 1918. He received his B.S. degree at Oklahoma State University, and his M.S. and Ph.D. degrees from West Virginia University. Before coming to New Mexico in 1956, Dr. Black was Head of the Animal Husbandry Department at Texas Technological College from 1954 to 1956. Prior to this, he was a member of the animal husbandry staff at West Virginia University for 14 years, and during 1953–54 was Assistant Dean and Director of the College of Agriculture, Forestry, and Home Economics at West Virginia University. He served in the U. S. Navy as a Lieutenant in the American and Pacific theaters during World War II.

Dr. Black's warm, friendly nature, fine mind and sound judgment made him a brilliant administrator, appreciated by his colleagues and all people in agriculture. At the time of his death he was secretary of the experiment stations section of the Land Grant College Association, member of the experiment station committee on organization and policy, chairman of the advisory committee, Forest and Range Experiment Station, Albuquerque, N. M., in addition to being a member of numerous other campus and agricultural committees.

With all his administrative duties, Dr. Black retained a keen interest in all phases of the livestock industry. He was the author of many bulletins and journal articles in animal husbandry. His services as a judge of livestock or meats were in wide demand at numerous state and regional fairs and national livestock shows. There was nothing he loved to do more than judge, but other demands on his time required a reduction in such activities in recent years. Because of his unusual talents as a speaker, he was often asked to preside or talk at meetings of agricultural and civic organizations. Such requests were never declined if they could be fitted into a busy schedule.

He was a member of Sigma Xi, Alpha Zeta, Fellow of the American Association for the Advancement of Science, and member of American Society of Animal Production.

He is survived by his wife, Theodora, and four sons, Baxter, Robert, John, and Stephen, all of Mesilla Park, N. M.

S. Orestes Bond

S. O. Bond was born at Hackers Creek in Upshur County, West Virginia on August 12, 1877. He received an A.B. degree from Salem College and West Virginia University, a Master's from Columbia and a Ph.D. from Alfred University. Dr. Bond served in many capacities in the elementary and secondary school systems in West Virginia until 1914 when he became Acting President of Glenville State College. He was appointed President of Salem College in 1919 and served in this capacity until 1951 when he became President Emeritus of that institution. Dr. Bond was one of the oldest breeders of purebred Hereford cattle in West Virginia and at the time of his death it was believed that he was one of the oldest breeders in the Eastern United States. He was a member of the firm, E. H. Bond and Brother, which established a purebred herd of Hereford cattle in 1901. This firm continued until 1937 and during the intervening years the production of the herd made very worthwhile contributions to the purebred and commercial industry in the Eastern part of the United States. In 1937 the firm was reorganized and its name changed to Bonds' Greenacres which is located at Jane Lew, West Virginia. The herd continued to make its contributions to the cattle industry and continued to function until Dr. Bond's death.

In addition to Dr. Bond's reputation as an educator and a breeder of Hereford cattle, he was also a very staunch supporter of the Seventh Day Baptist Church and a very active member of Kiwanis. Dr. Bond was held in highest regard as an educator, livestock breeder and a Christian gentleman. His sympathetic understanding of the problems of others, his willingness to give unstintingly of his time, energy and money to the betterment of youth, made him innumerable friends who will sorely miss him.

Dr. Bond died on January 11, 1959.

Raymond M. Crown

Raymond M. Crown, Associate Professor of Animal Industry at Louisiana State University, passed away July 7, 1960. He was born in Gainesville, Florida, August 13, 1901. He received his B.S. degree in Agriculture from the University of Florida in 1926 and M.S. degree in 1940.

As a University of Florida Experiment Station researcher he worked for 11 years as Agronomist and for 4 years as Assistant Animal Husbandman. In 1940 he was appointed as Agricultural Extension Swine and Meat Specialist at Louisiana State University. In 1946 he joined the teaching staff as Associate Professor in Animal Industry and Animal Husbandry of the Louisiana State Experiment Station which he held until his death.

All of his life he maintained an active interest in Animal Industry work and related fields. He was especially interested in animal production and meats, as a teacher, as a researcher, and extension worker. He has to his credit many publications in his field of interest, had a host of friends, and was known as the campus "Barbeque King".

Professor Crown is survived by his wife Pauline, who now resides in Weissport, Pennsylvania, and his son Ray, who is a Captain in the Army, stationed in California.

Henry E. Dvorachek

Henry E. Dvorachek, former Head of the Department of Animal Industry, University of Arkansas,

died October 2, 1960 at Washington County Hospital in Fayetteville.

He was born July 27, 1885, Glencoe, Minn., and received his B.S. degree in Agriculture, University of Minnesota in 1910. He joined the Department of Animal Husbandry Staff, Colorado State University in 1910 and in 1915 he was appointed Head of the Department of Animal Industry, University of Arkansas. He served in this position until June 30, 1931, when he resigned to become manager of the Ozark Dairy Products Company, in Fayetteville.

He was employed by the Farm Security Administration from 1934 until his retirement in 1947. He remained an active worker in Fayetteville church and civic organizations. He was a member of the American Society of Animal Production, The American Dairy Science Association and a co-founder of the Arkansas chapter of Alpha Zeta.

He is survived by his wife Clara, a son William H., a daughter (Mrs.) Mae Ellen Bruce and six grandchildren.

August B. Salmela

August B. Salmela, Assistant Professor of Animal Husbandry at the University of Minnesota's North Central School and Experiment Station at Grand Rapids died on December 10, 1959, in Duluth, Minnesota.

Professor Salmela was born on a farm in St. Louis County near Embarrass, Minnesota, on January 7, 1914. He grew up in Northeastern Minnesota, attending public schools there, later graduating from the North Central School of Agriculture at Grand Rapids and from the College of Agriculture, Forestry, and Home Economics of the University of Minnesota. He also received his Master of Science and Doctor of Philosophy degrees from the University of Minnesota.

He began his career with the University as a research assistant, later becoming an Instructor and on July 1, 1959, was promoted to the rank of Assistant Professor. Always interested in the development of good livestock, he directed his attention to the dairy cattle improvement at the North Central Experiment Station. His primary research, however, centered on the development of economically produced meat type hogs. It was while carrying out a segment of that research in Duluth that he was stricken with a heart attack and passed away.

He was an enthusiastic teacher and many students throughout Northern Minnesota will remember him not only for his subject matter, but also for his deep personal interest in them.

Professor Salmela served in the United States Army during World War II. In 1949 he visited Brazil for the International Basin Economy Corporation. He worked for a private swine breeding establishment in Indiana just prior to his first full time appointment at the University.

He is survived by his wife Viola and three children, Julie, Linda and Bruce, who live in Grand Rapids, Minnesota.

Philip E. Skinner

Philip E. Skinner passed away on June 25, 1960, following an illness of several months.

Philip was born in Brookings, South Dakota on December 10, 1924. He received the Bachelor of Science degree in Agriculture from the University of Nebraska in 1949 and was married the same year to Phyllis Schinzel of Grafton, Nebraska. He was engaged in farm and ranch work in Western Nebraska until he entered graduate school at Oklahoma State University in September 1956. He received the Master of Science degree from this institution and had completed much of the work toward the Ph.D. degree in Animal Breeding. At the time of his death, Phil was employed as a research associate by the Cancer Eye Study Section of the M. D. Anderson Hospital at Houston, Texas, and he was living at the Ft. Reno Livestock Research Station at El Reno, Oklahoma.

Phil was an active worker in the Methodist Church, Farmhouse Fraternity, Alpha Zeta and Sigma Xi. Survivors include his wife Phyllis, two sons, Mike and Tom, and a daughter, Jennie.

Stanley Wallace Terrill

Stanley W. Terrill, Head of the Swine Division of the Department of Animal Science, University of Illinois, died July 23, 1960, at Champaign, Illinois.

Born near Redfield, Iowa, on January 21, 1919, Dr. Terrill was graduated with a B.S. degree from Iowa State College, where he received many honors for his scholarship and extracurricular activities. After serving as a County 4-H Club Agent in Warren County, Iowa, for one year, he came to the University of Illinois to pursue graduate study in 1941.

His work at Illinois was interrupted by World War II, during which he served as a Radar Technical Officer in the United States Navy with the rank of Lieutenant. Following discharge from service in 1946, he returned to the University of Illinois to continue studies for the Ph.D. degree in Animal Science. After receiving his degree in 1948, Dr. Terrill was retained on the staff of the Department of Animal Science. In 1950, he was made Head of the Swine Division of the Department.

Stan's many contributions to the field of swine nutrition and management were made through his enthusiastic and thorough teaching of graduate and undergraduate students and his outstanding research ability. In addition, he had the rare ability of interpreting fundamental research for the solution of practical problems. For this reason, he was widely sought as a speaker by various agricultural groups. His research included studies on the protein and vitamin requirements of swine, the value of antibiotics, unidentified factors and drugs in swine nutrition, and the breeding and management aspects of swine production. His ability to evaluate research in his field led to his appointment to the Editorial Board of the Journal of Animal Science.

He was a member of the American Society of Animal Production, American Association for the Advancement of Science, Animal Nutrition Research Council, Alpha Zeta, Gamma Sigma Delta, and Sigma Xi. In the community, he was a very active member of the McKinley Presbyterian Church.

He is survived by his wife, Vivian, and three children, David, Marilyn, and Nancy.

His untimely death is a real loss to the Department of Animal Science and to the swine industry of the United States. He will be remembered for his sincerity, his friendly and warm personality, and his promotion of better teaching and research in his chosen field.

Henry G. Zavoral

Henry G. Zavoral, 65, former Professor and Extension Animal Husbandman at the University of Minnesota, died of a cerebral thrombosis Friday, January 8, 1960, at Midway Hospital, St. Paul. He had been ill since December, 1959.

He was known around the state as an avid proponent of "meat type" hog production. He had promoted systems of raising and caring for swine that are now widely adopted, and was himself a noted breeder of Duroc hogs. He was also popular as a state fair swine show superintendent, show ring judge, and moderator.

Mr. Zavoral was born in McLeod county, April 2, 1894, attended the University of Minnesota, and served with the U. S. Army Veterinary Service in France during World War I. He served as Nobles county agent from 1918–21, then came to the St. Paul Campus as Extension Animal Husbandman, a position he held until his retirement in early 1959.

In the early years, he organized colt and stallion clubs and conducted demonstrations around the state on how to hitch large numbers of horses to a single load.

When frozen food lockers came into use, he gave hundreds of demonstrations on their use and value.

In 1930, Mr. Zavoral went to Russia for a year as American adviser on livestock production for the Soviets' first 5-year plan. While in Berlin, Germany, to buy breeding stock for the Russians, he was joined by Angeline Keenan, his fiancee from Minneapolis. The couple was then married on Berlin's Unter Den Linden.

During the 30's, Mr. Zavoral's interest turned to hogs, and he saw a need for changed ways of hog farming. He noted that people no longer wanted as much fat as they used to get from the 300-pound and heavier hogs of earlier years. So, in cooperation with county agents, he helped farmers conduct meat-type hog demonstrations, evaluate carcasses of leaner hogs, and adopt better swine raising practices. He wrote a bulletin "Hog Health Makes Wealth" which has been used widely by agents and farmers around the Midwest.

Mr. Zavoral was superintendent of the National Swine Show at Peoria in the early 20's and directed the Junior Livestock Show at South St. Paul from 1924–30. For 12 years, he was moderator for the National Barrow Show at Austin and since 1955 was superintendent of the swine show at the Minnesota State Fair. He organized the first Spring Barrow Show at Albert Lea in 1945 and helped set up many district barrow shows. Starting 16 years ago, the University, under Mr. Zavoral's supervision, annually named a "swine honor roll", a group of farmers who have shown top efficiency in hog production.

Mr. Zavoral is survived by his wife and son, James.

CHAIRMEN OF COMMITTEES, PROGRAM CHAIRMEN AND SPECIAL ASSIGNMENTS FOR 1961

Standing Committees

Research ..... K. E. Gregory
Extension ..... J. S. Robinson
Membership ..... T. J. Cunha
Teaching ..... J. J. Kiser
Career Information ..... W. A. Cowan

Special Committees

Information ..... Carl F. Neumann
Necrology ..... F. S. Willson
Nominations ..... W. P. Garrigus
Registration and Facilities ..... R. J. Meade
Resolutions ..... A. L. Harvey
Special Grants for Foreign Travel and Study ..... J. O. Grandstaff
Morrison Award ..... T. J. Cunha
American Feed Manufacturers' Award ..... O. G. Bentley
Fellows ..... Warren Gifford
Extension Award ..... J. K. Loosli
Teaching Award ..... Floyd Pahnish

Program Committees

Breeding and Genetics ..... R. E. Comstock
Meats ..... A. M. Mullins
Nutrition ..... A. L. Neumann
Pastures and Forages ..... O. C. Hall
Physiology ..... R. H. Dutt
Livestock Management and Housing ..... J. E. Burnside

Special Representatives

American Association for Advancement of Science Council ..... T. C. Byerly
Committee on Intersociety Cooperation and Scientific Manpower Commission ..... J. E. Foster
Division of Biology and Agriculture, National Research Institute ..... C. F. Sierk
European Association for Animal Production ..... N. R. Joshi
Ralston Purina Award Committee ..... T. J. Cunha
Pasture and Forage Crop Improvement Committee ..... W. B. Anthony
American Grassland Council ..... P. G. Woolfolk

NEWS AND NOTES

Proposed amendments to the Constitution to be voted on at the 1961 meeting of the Society:

ARTICLE I. THE SOCIETY AND ITS PURPOSES

Section 1. Name.

Change from "This association shall be known as the American Society of Animal Production" to "This association shall be known as the American Society of Animal Science".

ARTICLE IV. ELECTION OF OFFICERS

Section 1. Procedure.

Change from "The election of officers shall be held at the annual meeting of the Society. A nominating committee shall have been appointed by the president and shall have nominated not less than two candidates for vice-president and, before expiration of the three-year term of office, not less than two candidates for secretary-treasurer. When the secretary-treasurer after his term of office normally is to succeed to the vice-presidency, the committee may put only his name in nomination. The slate of nominees shall be published in the current year's November issue of the Journal of Animal Science. Additional nominations may be made from the floor at all annual meetings. Election shall be by ballot. A majority of the votes shall be required for election. In case an annual meeting is not held, election shall be by mail." to, "The election of officers shall be by mail ballot just prior to the annual meeting. A nominating committee shall have been appointed by the president and shall have nominated not less than two candidates for vice-president and before expiration of the three-year term of office, not less than two candidates for secretary-treasurer. When the secretary-treasurer after his term of office normally is to succeed to the vice-presidency, the committee may put only his name in nomination. The slate of nominees shall be published in the current year's August issue of the Journal of Animal Science, or at least the issue prior to the one in which the abstracts for the annual meeting appear. The card ballot distributed by the Secretary shall provide space for write-in candidates. To be counted the card ballots must be received by the Secretary not later than three days prior to the first day of the annual meeting. The ballots will be counted by a Committee of Tellers at the time of the annual meeting. A majority of votes shall be required for election."

Back numbers of the Journal of Animal Science or Proceedings of the Society are available for purchase from the Business Manager as noted in his report in the minutes of the Annual Meeting elsewhere in this issue. Numbers of the Journal other than those listed become available occasionally and specific inquiry should be made for any numbers wanted which are not listed in the above report. Reprints of review papers from the 50th Anniversary Issues are also available for $2.00 each.

Ralston Purina Research Fellowship Awards Program for the 1961–62 School Year. Again this year, ten Fellowships in the amount of $2000 each will be granted for graduate study in Agricultural Research. Selection of the recipients will be made by the following committee, chosen as representatives by the Associations indicated: W. A. Hagan, National Animal Disease Lab. (Ames, Iowa), American Veterinary Medical Association; Robert MacVicar, Oklahoma State University, American Association of Land Grant Colleges and State Universities; T. J. Cunha, University of Florida, American Society of Animal Production; R. H. Thayer, Oklahoma State University, Poultry Science Association; N. L. Jacobson, Iowa State University, American Dairy Science Association. Application blanks for these Fellowship Awards may be obtained by writing the Ralston Purina Research Awards Committee, % Mr. J. D. Sykes, Ralston Purina Company, St. Louis 2, Missouri.

Training Program for Steroid Biochemistry. Under the sponsorship of the National Cancer Institute, N.I.H., U.S.P.H.S., specialized programs have been established in Worcester and Salt Lake City to train investigators in the theoretical and methodological aspects of the biochemistry of steroids and related compounds. Each program includes lectures, planned laboratory work, and research. Postdoctoral candidates (M.D. or Ph.D.) selected for admission both at Worcester and Utah will receive stipends of $5500 for a one-year period. Predoctoral candidates (B.S., M.S., or equivalent) selected for admission (only at Worcester) will receive stipends of $1800 for a six-month period. Send inquiries and requests for applications to: Dr. Kristen Eik-Nes, University of Utah, Salt Lake City, Utah, or Dr. William R. Nes, Clark University, Worcester, Massachusetts. Classes start October 1, 1961. Final date for completion of applications is June 1, 1961.

The National Science Foundation announces that March 1, 1961 is the next closing date for receipt of proposals in the Graduate Laboratory Development Program. Proposals received after March 1 will be reviewed following the next closing date, September 1, 1961. This program requires at least 50 percent participation by the institution with funds derived from non-Federal sources. Proposals, as well as requests for additional information, should be addressed to: Office of Institutional Programs, National Science Foundation, Washington 25, D. C.

Summer Institute for College Teachers of Statistics. The National Science Foundation will sponsor a Summer Institute for College Teachers of Statistics at Iowa State University for the 11-week period from June 5 through August 18, 1961. The Departments of Statistics of three other universities, Kansas State, Utah State and the University of Wyoming, are cooperating with Iowa State's statistical center in presenting this institute. Financial support in the form of stipends, dependency allowances and travel allowances will be awarded to 50 eligible applicants.

All American college and university teachers who are, or who during the 1961–62 academic year will be, required to teach one or more courses in statistics as part of their regular assignments are eligible for consideration. The institute is planned to provide additional basic training in statistics for present and prospective teachers who, though well-grounded in other fields, have limited backgrounds in statistics. Also it will provide more advanced courses and seminars designed to keep college and university teachers abreast of new developments. Courses are scheduled in Statistical Methods, Theory of Statistics, Experimental Design, Survey Designs, Topics in Foundations of Probability and Statistics, and Intermediate Applied Decision Theory. In addition, an opportunity will be provided for those interested to observe a demonstration class in Principles of Statistics at the undergraduate level. The faculty will include the institute director, Dr. T. A. Bancroft, director of the Iowa State University Statistical Laboratory and head, Department of Statistics; Dr. R. J. Buchler, associate professor of statistics, Iowa State University; Dr. H. T. David, associate professor of statistics, Iowa State University; Dr. H. C. Fryer, head of the Department of Statistics and Statistical Laboratory director, Kansas State University; Dr. H. O. Hartley, professor of statistics, Iowa State University; the institute associate director, Dr. D. V. Huntsberger, associate professor of statistics, Iowa State University; and Dr. R. L. Hurst, head of the Department of Applied Statistics and Statistical Laboratory director, Utah State University. Guest lecturers will present a series of special seminars. Requests for information or application forms should be addressed to: The Director, Summer Institute in Statistics, 102 Service Building, Iowa State University, Ames, Iowa.

Copies of the Proceedings of the Symposium on Magnesium and Agriculture held at West Virginia University on September 3rd and 4th, 1959, are now available at $2.00 per copy, and may be obtained by writing to Dr. D. J. Horvath, Department of Animal and Veterinary Science, West Virginia University, Morgantown, West Virginia.

Grants for International Meetings: The National Science Foundation makes grants to scientists in support of attendance at a limited number of international scientific meetings each year. Main criteria for selection of these meetings are (1) must be of broad scientific interest; (2) give substantial emphasis to basic research. Applications for travel grants are evaluated on the basis of scientific merit, taking into account the research record of the applicant, especially with respect to basic research, his prospective contributions to the meeting (scientific papers, chairman of section, etc.), and possible values to his and other research in the U. S. resulting from attendance. Grants to individuals generally cover only the cost of coach airline fare from point of origin to destination and return or its equivalent. Per diem and other expenses incident to the trip are not included.

Applicants for travel grants may be submitted on NSF Form 9–1, Application for Grant in Support of Attendance at International Scientific Meetings, which can be obtained from National Science Foundation. Or to save time the application can be made by letter giving the following information:

APPLICATION FOR GRANT IN SUPPORT OF ATTENDANCE AT INTERNATIONAL SCIENTIFIC MEETINGS

(Submit two copies)

I. General Information

1. Name of applicant
2. Date of birth
3. Department
4. Institution
5. Address of institution
6. Mailing address, if different from above.
7. Education—Degrees and Institution
8. Experience—major positions held
9. Previous professional visits abroad within past 5 years. Where? When?
10. Publications—list more important ones—not more than 5

II. International Scientific Meeting to be Attended

1. Name of meeting or meetings
2. Inclusive dates of meetings
3. What other contacts do you intend to make?
4. Copy or facsimile of official announcement of meeting, program or other relevant information

III. Purpose of Attendance

1. Official representative of scientific society, government agency, or other organization—give name
2. Member of national or international commission —give name
3. Will you present a scientific paper, or otherwise participate? If yes, give title of paper or indicate nature of participation
4. Other purposes (specify)
5. Relationship of meeting and contacts to your own research or scientific interests

IV. Budgetary Estimates

1. Proposed itinerary
2. Estimate of those travel costs which are requested from the National Science Foundation.
   a. Travel in USA .....
   b. Oceanic travel—sea .....
   air .....
   c. Travel in foreign countries .....
   d. Other (itemize) .....
3. Other travel funds available to you during calendar year in which meeting occurs (e.g., institutional allowance, research grants)
   Source ..... Amount .....
   ....
   ....
4. Tentative dates of departure and return
5. Signature of applicant

Approximately one month is required for evaluation of applications. Two additional months are generally required for processing applications that have been approved. Review of applications for a particular meeting is facilitated by having them submitted to NSF at one time. It is suggested that applications to NSF for travel grants be sent to James O. Grandstaff, State Experiment Stations Division, ARS, USDA, Washington, D. C., who will transmit them as a group to NSF. In selected cases the American Society of Animal Production may make application to NSF for travel grant, and would have responsibility for screening applications and making recommendations

to NSF on awards. Society members will be informed when this procedure is to be used.

Charles E. Kellogg, formerly head of ARS research on fur bearing animals and more recently in foreign visitors' training work, retired on October 31 after 33 years Government service.

M. W. Hazen, project leader, Beef Cattle Research, USDA, retired August 31. Walter L. Reynolds, Animal Husbandman, USDA, was appointed effective September 12, to replace Mr. Hazen. Dr. Reynolds received his BS from New Mexico State University and his M.S. and Ph.D. degrees from the University of Florida.

Tony Fellhauer, Wyoming agricultural extension livestock specialist for 22 years, retired November 1. Mr. Fellhauer, well known for his long service to the livestock industry, received the Record Stockman magazine's "Man of the Year in Livestock" award in January 1958—the first winner who was not a rancher or feedlot operator.

Glenn H. Beck, Director of the Kansas Agricultural Experiment Station, was appointed as Dean of Agriculture on November 16. He succeeds Dean Arthur D. Weber. Dean Beck is nationally recognized for his research in dairy nutrition and is also a well-known judge of dairy cattle.

J. T. Miles, B.S. '40, M.S. '48, U. of Tennessee; Ph.D. '51, University of Wisconsin, has been named Head of the Dairy Department, University of Tennessee, to succeed Prof. C. E. Wylie, who retired August 31.

Fred F. McKenzie has resigned from the staff of the Department of Dairy and Animal Husbandry, Oregon State College and accepted an ICA appointment. He is now with the Kentucky Contract Team, University of Indonesia, P. O. Box 54, Bogor, Java, Indonesia. Dr. McKenzie is chairman of the Department of Veterinary Medicine and Animal Science.

I. R. Jones is on a Fulbright Lectureship at the University of Ireland, Cork, Ireland, for the school year 1960–61.

Ray Kliewer who holds B.S. and M.S. degrees from Oregon State College has joined the staff of the Department of Dairy and Animal Husbandry at Oregon State College as Instructor in the Dairy Division.

Hugo Krueger, Physiologist, Department of Dairy and Animal Husbandry, Oregon State College, is on a two-year assignment under contract with the University of Wyoming in Afghanistan. His address is: American Embassy, Kabul, Afghanistan, % Department of State, Washington 25, D. C.

Floyd Stout, Ph.D., Oregon State College 1960, has joined the staff in the Nutrition Section.

Jay L. Lush of Iowa State University received from the Deutsche Gesellschaft fur Zuchtungskunde the Hermann von Nathusius medal for scientific contributions to practical animal husbandry. The presentation was at Hannover on September 19 at a reception given by the Minister of Agriculture of Lower Saxony to the lecturers and students of the NATO-sponsored short course at Mariensee for European research workers in animal husbandry. The medal was established in 1928 at the instigation of Professor C. C. Kronacher in honor of Hermann von Nathusius, who is generally regarded as the founder of scientific animal husbandry in Germany.

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INFORMATION FOR CONTRIBUTORS

1. Papers may be submitted reporting work whose ultimate objective is clearly the solution of a livestock production problem and whose contents should be of interest particularly to those dealing with livestock production problems. Review papers on subjects of general interest will also be published at intervals. These will be prepared on invitation of the Editorial Board.

2. All papers will be subject to critical review by the Editorial Board, or others designated by the Editor. Papers needing revision will be returned to authors and should be revised and returned promptly. Papers not suitable for publication will be returned to authors with a statement of reasons for not accepting them.

3. Papers submitted should be based on new and adequate information. The primary purpose of a scientific publication is to provide information based on well-described data and to exchange ideas. Authors are therefore discouraged from submitting for publication results of research involving products whose ingredients cannot be disclosed. Information on composition and source of any product which may have a bearing on the experimental results should be provided. Authors are also discouraged from submitting papers based on routine product testing.

4. Consult a recent issue for style and form. The following are guides:

   (a) Manuscripts must be typed, double-spaced and the ribbon copy submitted. Submission of two copies (one carbon) will facilitate review.

   (b) All pages, including those of tables and legends, must be numbered. The lines of the text, but not of tabular material or "Literature Cited", must be numbered in the left margin, beginning with one at the top of each page.

   (c) Tables should be as few and as simple as is feasible for presentation of the essential data. Each table should be on a separate sheet.

   (d) The legend for each figure should be typed on a separate sheet. Photographs should be carefully prepared and labeled. Magnification of photomicrographs should be indicated on legends. Charts should be prepared in India ink. Typed material on charts is not acceptable. Material that will not reproduce satisfactorily will be returned for revision. For suggestions on preparing illustrative material see the "Style Brief" published by the Wistar Institute Press, Philadelphia. If in doubt, consult the Editor before putting material in finished form.

   (e) The introduction should consist of no more than 200 words indicating very briefly the nature of the questions asked and the reasons for asking them. Most of the relevant literature should be mentioned later in the discussion of the results. Concise presentation of data is desired and there should be evidence of statistical examination wherever obviously useful in the interpretation. Unsupported hypotheses should be avoided. The organization of a manuscript may vary with the nature of the material, but manuscripts should contain a statement of application of the results or their relation to some problem of the livestock industry. Each paper should end with a summary in the form of an abstract containing the most pertinent data in the briefest possible form.

   (f) Literature citations must be listed in alphabetical order and should be referred to in the text by author and date, i.e., Castle (1924) or (Castle, 1924); Best *et al.* (1951) or (Best *et al.*, 1951). Gitations may be referred to by number in review articles where the number of citations is too great to permit convenient use of the above form. "Literature Cited" lists should be typed in a style used in a current issue of the JOURNAL.

5. Manuscripts will be edited in the order received, and accepted papers will be published as nearly in this order as feasible.

6. There will be a charge for making engravings in excess of the equivalent of one column per paper and of printing tables in excess of the equivalent of two columns per paper. The author will be asked when his manuscript is accepted to indicate the institution, agency or individual responsible for the charges and for the reprints ordered at that time.

7. Authors will receive galley proof of articles together with a form for supplying an abstract for "Biological Abstracts". *Corrected proof and abstract should be mailed to the Editor within three days after they are received. Failure to do this may result in delay of publication until the next issue.*

8. One author of each manuscript should be a member of the Society. Papers by non-members can be published only after approval by the Executive Committee.

9. A "News and Notes" section will be published in each issue, including Society announcements, changes in positions and other items of professional interest to members. All such items should be sent promptly to the Editor.

10. Abstract of papers to be presented at the annual meetings of the Society will be published in the November issue. Such abstracts must not exceed 250 words in length, should be typed (double spaced) and the ribbon copy submitted, the lines on the page should be numbered, in the left margin, from top to bottom, and must be in the hands of the Secretary of the Society by August 15, preceding the meeting. These abstracts must be summaries of results and conclusions. They should be checked carefully before submission—proofreading of the galleys will usually not be possible.

11. Presentation of a paper at the annual meeting does not exclude it from publication in complete form in the JOURNAL. Such papers may be submitted to the Editor and will be given equal consideration with others submitted.

12. All manuscripts and correspondence concerning them should be addressed to the Editor: A. B. Chapman, Department of Genetics, University of Wisconsin, Madison 6, Wisconsin, or to the publisher: Boyd Printing Company, Inc., 49 Sheridan Ave., Albany 10, N. Y.

13. All correspondence concerning subscriptions to the JOURNAL OF ANIMAL SCIENCE and other business matters should be addressed to the Business Manager, C. E. Terrill, Animal Husbandry Research Division, Agricultural Research Center, Beltsville, Maryland, or to the publishers, Boyd Printing Company, Inc., 49 Sheridan Avenue, Albany 10, N. Y.

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